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Diversity of *Phytophthora* Species in Canterbury Waterways

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science.

Diversity of *Phytophthora* species in Canterbury Waterways

by

Ashika Ashnita Prasad

Spread worldwide, the genus *Phytophthora* includes a diverse group of pathogens that affect a wide range of agricultural crops and plants in native ecosystems. Water surveys have become popular in regions where early detection of infected areas is important for containment and eradication of *Phytophthora* spp., with more than 20 new *Phytophthora* spp. isolated from waterways around the world. This study identified the best method and leaf bait for *Phytophthora* recovery which was used for a more extensive *Phytophthora* diversity study in Canterbury, New Zealand, waterways. Additionally, four methods for screening pathogenicity of *Phytophthora* spp. recovered from the waterways was also done to identify a rapid assay that could identify pathogenic isolates.

Three methods (laboratory baiting, river baiting (*in situ*) and filtration method) were evaluated for *Phytophthora* recovery from two sites in the Halswell River in summer (February 2018). *Phytophthora* baiting was done by floating leaf baits in water samples (laboratory baiting) or waterways (river baiting) for 7 days after which the lesions on the leaf baits were cut and cultured on *Phytophthora* selective media for *Phytophthora* isolation. For filtration method membrane filters (3-µm pore size) was used to filter water samples and the membrane filters cultured on *Phytophthora* selective media for isolation of *Phytophthora* isolates. The study identified water baiting, either laboratory baiting or river baiting as being suitable to study of *Phytophthora* spp. diversity in waterways. Filtration method was not effective due to high *Pythium* recovery which contaminated the *Phytophthora* colonies. Additionally, seven leaf baits (*Banksia attenuata, Camelia japonica*, *Cedrus deodara, Pinus radiata, Pittosporum undulatum, Pittosporum eugenioides* and *Rhododendron arboreum*) were evaluated for *Phytophthora* recovery. *Rhododendron arboreum* recovered the highest number of *Phytophthora* isolates and species. As identified in this study, future *Phytophthora* diversity studies should focus on using laboratory baiting and river baiting methods with *R. arboreum* leaf bait.

The laboratory baiting method was used for the *Phytophthora* diversity study (autumn baiting; May 2018) using *Ce. deodara, Pi radiata and R. arboreum* leaf baits in six waterways (Selwyn River (Waikirikiri), Ashburton River (Hakatere), Prices Valley River, Kaituna Valley River, Halswell River

and Lake Hood) with a total of 25 sites. *Phytophthora lacustris* was the most commonly isolated species, followed by *Ph. gonapodyides*, *Ph. chlamydospora* x *Ph. amnicola* hybrid and *Ph. thermophila x amnicola* hybrid. *Rhododendron arboreum* was found to be more effective at isolating *Phytophthora* spp. over a range of water pH, and again recovered the highest number of *Phytophthora* isolates and species. This study reports the first recovery of some species in the Canterbury region and includes; *Ph. thermophila x amnicola* hybrids, *Ph. chlamydospora* x *Ph. thermophila* hybrids, *Ph. amnicola x Ph. chlamydospora* hybrids, *Ph. chlamydospora, Ph. bilorbang, Ph. lacustris,* and *Phytophthora* sp. LS-2018c strain CL181. *Phytophthora gonapodyides* and *Ph. cryptogea* were also recovered however, these species have been previously reported to be widely spread throughout New Zealand. *Phytophthora cactorum* has mostly been found in association with orchards in New Zealand, however this study reports the first isolation from waterways. *Phytophthora* sp. LS-2018c strain CL181 and *Ph. thermophila* are new species in New Zealand identified through this study. The study also identified seasonal difference in *Phytophthora* spp. diversity in the two sites in the Halswell River stressing the need to carry out *Phytophthora* spp. diversity studies over all seasons.

Pathogenicity of three commonly recovered *Phytophthora* spp. from Canterbury waterways, *Phytophthora* sp. LS-2018c strain CL 181, *Ph. chlamydospora* and *Ph. gonapodyides,* was evaluated using four methods. Testing *Phytophthora* isolates using agar plugs on lupin seedlings grown on sterile paper towels was identified as a good rapid assay for determining the pathogenicity of *Phytophthora* isolates recovered from waterways, with results observed 4 days post inoculation. All three *Phytophthora* species isolates were found to be pathogenic on lupin seedlings. *Phytophthora* isolates that are shown to be pathogenic in the screening assay should be included in further pathogenicity test using crops, fruit trees, native and exotic tree species to evaluate the risks these species pose to New Zealand's agricultural and native ecosystems.

Overall, recovery of new *Phytophthora* spp. in Canterbury waterways has provided new insights on the importance of carrying out *Phytophthora* surveillance in waterways. Additionally, this study provides recommendations on improvements for future *Phytophthora* diversity studies.

Keywords: *Phytophthora,* Canterbury waterways, recovery method, leaf baits, pathogenicity, seasonal variation, abiotic factors

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Chapter 1: Introduction

New Zealand has a thriving agricultural and forestry sector which is constantly threatened by pests and diseases, including pathogens belonging to the *Phytophthora* genus (Scott & Williams, 2014). New Zealand's flourishing forest resource cover over 8 million hectares (ha) representing 29% of the total land area out of which 6.3 million ha are indigenous forests and 1.7 million ha of exotic forest (Stats NZ, 2005). The indigenous forests include species such as beech, kauri, tawa, taraire and rimu (Stats NZ, 2005). While the exotic forest on which the forestry industry is heavily dependent upon is made of 90% of radiata pine (*Pinus radiata*) and the remaining forests containing Douglas fir (*Pseudotsuga menziesii*), eucalypts and other species (Ministry of Primary Industries, 2017). The forestry sector contributes an annual gross income of $5 billion contributing 3% of the GDP. Additionally, the sector employs around 20,000 people (Ministry of Primary Industries, 2017). Therefore, New Zealand's native and exotic forests play an important economic, ecological, environmental and social function (Scott & Williams, 2014). Apart from forests, *Phytophthora* spp. are also known to attack other native ecosystems, and agriculture and horticultural production (Scott & Williams, 2014).

Around 75% of land is used for agriculture in the Canterbury region (Stats NZ, 2005) however, there are no current data available on the *Phytophthora* spp. present. Changes in land use, climate change and anthropogenic activities can cause outbreaks of *Phytophthora* diseases that can be detrimental to the region. Therefore, water surveillance of major waterways in close proximity to agricultural land that drain into the system can provide information on the presence of *Phytophthora* spp. It will also help identify any new species or hybrids for control and eradication programs.

1.1 The Genus *Phytophthora*

The genus *Phytophthora* contains mostly plant pathogens that are either host specific or have a broad host range, with many new *Phytophthora* spp. identified from forests around the world in the last decade (Huai *et al.*, 2013). Spread worldwide, the genus *Phytophthora* includes a diverse group of pathogens that affect a wide range of agricultural crops, forestry and plants in native ecosystems (Rahman *et al.*, 2014; Yang *et al.*, 2017), with a total of 124 described species (Lamour, 2013). *Phytophthora* spp. require aquatic environments to maintain their asexual life cycle and for dispersal (Hüberli *et al.*, 2013; Redondo, 2018; Stamler *et al.*, 2016a). With the increasing number of species isolated from streams and forests, *Phytophthora* are hypothesized as being waterborne opportunistic pathogens, canker or fine-root pathogens and foliar pathogens (Jung *et al.*, 2018b; Redondo *et al.*, 2018). Although most *Phytophthora* spp. are reported to be primary plant pathogens however, species from *Phytophthora* clade 6 and clade 9 have a saprophytic lifestyle in aquatic environments and are opportunistic pathogens (Jung *et al.*, 2018b). Over the last decade the number of recognised *Phytophthora* spp. have doubled with new species added on a monthly basis (Brouwer *et al.*, 2012)

and this has mainly been attributed to the application of molecular tools that have distinguished the identity of closely related species (Thines, 2013).

Many *Phytophthora* spp. are soil borne pathogens and produce several different structures that enable dispersal and infection of host plants. These structures include the asexually formed spores, zoospores; sporangia and chlamydospores, while oospores are the sexually produced spores (Drenth & Guest, 2004). All the spores are capable of causing infection while chlamydospores and oospores overwinter or are the resting structures (Drenth & Guest, 2004). Homothallic (self-fertilizing) *Phytophthora* spp. are able to produce oospores rapidly and in large quantity, while heterothallic (requiring cross fertilization) species such as *Ph. cinnamomi* can only produce gametangia in response to chemical stimulation from the opposite mating type (Drenth & Guest, 2004). Therefore, sexual reproduction occurs when the A1 and A2 mating types are adjacently growing together and the fertilization of the female organ known as the oogonium by an antheridium which is the male organ to form oospores (*Phytophthora* basics, 2017). Oospores are thick- walled with a globose structure that allows long-term survival in plant tissue and soil (*Phytophthora* basics, 2017). Oospores and chlamydospores germinate to produce sporangiophores that produce sporangia (*Phytophthora* basics, 2017). The chlamydospores can germinate to form mycelium, sporangium or secondary chlamydospores depending on the availability of water and nutrients (Weste & Marks, 1987). Sporangia formed on debris and roots on the soil surface are washed into water pools, rivers, and creeks (Drenth & Guest, 2004). On different hosts, *Phytophthora* spp. cause various symptoms such as rot of fine feeder roots, root canker, wilt, stem canker, declining yield, collar rot, gum exudation, reduced fruit size and heart rot (Drenth & Guest, 2004).

Forty-three *Phytophthora* spp. have been reported in New Zealand including two new species., *Ph. pini* and *Ph. gregata* (Lewis, 2018; Manaaki Whenua Landcare Research, 2019d; Scott & Williams, 2014). *Phytophthora* spp. of concern in New Zealand include *Ph. cinnamomi,* which is associated with forestry and natural ecosystem causing kauri (*Agathis australis*) root and stem dieback; *Ph. agathidicida* identified as the primary cause of kauri dieback, soil-borne *Phytophthora* spp. causing kawakawa (*Macropiper excelsum*) dieback; while other species are associated with pine and *Eucalyptus* spp. (Scott & Williams, 2014). *Phytophthora cinnamomi, Ph. kernoviae* and *Ph. multivora,* which are present in New Zealand, cause diseases of great consequence in forests internationally (Scott & Williams, 2014) however, they are not yet as aggressive in New Zealand forests (Scott & Williams, 2014). Other *Phytophthora* spp. currently recorded as being present and associated with plant diseases in New Zealand are outlined in Appendix A.1.1. The origin of many *Phytophthora* spp. in New Zealand are unknown and 60% of the *Phytophthora* spp. affect agriculture; while 32% are associated with exotic forestry and 35% affect native ecosystems (Scott & Williams, 2014).

Phytophthora spp. co-evolve within plant communities however, exposure to new hosts and environmental changes present opportunities for the introduction of new host- pathogen combinations

and occurrence of sexual reproduction resulting in new species (Scott & Williams, 2014). According to Redondo (2018) outbreaks of invasive exotic *Phytophthora* spp. frequently have a long term effect on the host populations often resulting in high mortality. New *Phytophthora* spp. has been identified from broad surveys carried out in soil or streams in forest stands and *Ph. ramorum* is a good example of a newly identified species that is highly destructive to trees and ornamentals in the USA and Europe (Huai *et al.*, 2013; Werres *et al.*, 2001). The devastation caused by exotic *Ph. ramorum* and *Ph. alni* in Europe and north-western USA has resulted in a need for early detection of *Phytophthora* spp. from water, soil and plant samples (Hüberli *et al.*, 2013).

1.2 Different methods of *Phytophthora* isolation

1.2.1 Isolation of *Phytophthora* from waterways

Water surveys have become popular in regions where early detection of infected areas is important for containment and eradication of *Phytophthora* spp. (Hüberli *et al.*, 2013). More than 20 *Phytophthora* spp., including *Ph. ramorum* and *Ph. cinnamomi* have been identified from waterways around the world (Hüberli *et al.*, 2013). Commonly recovered from the waterways are the Clade 6 *Phytophthora* spp. which are hypothesised to have a prevalent saprophytic lifestyle (Hüberli *et al.*, 2013; Redondo, 2018; Reeser *et al.*, 2011; Stamler *et al.*, 2016a). According to Hwang *et al.* (2008), *Phytophthora* spp. are well adapted to an aquatic environment and water surveillance can be effectively used to survey large land areas that drain into a particular stream. Water surveillance can be used to determine the presence of *Phytophthora* spp. and their spread into different habitats such as agriculture, horticulture, forestry and native ecosystems. This can allow different disease management strategies to be implemented for control and eradication of *Phytophthora* pathogens. Water surveys can be done by baiting methods or filtering water samples which has been successful in *Phytophthora* spp. isolation (Ivors, 2018; Martin *et al.*, 2012).

1.2.2 Baits for *Phytophthora* isolation from waterways

Unwounded baits discourage bacteria and *Pythium* from colonising the bait, and leaf tissue bait is preferred over fruit of apple, pear or lemon (Martin *et al.*, 2012). A wide range of plant foliage such as camellia, rhododendron, and oak (*Quercus* sp.) has been used to attract diverse *Phytophthora* spp. (Martin *et al.*, 2012). Fruits such as green pear can also be used for baiting of *Phytophthora* from waterways, however fruit should not have a waxed surface nor fungicide application as this will affect baiting success. This method is also not selective for only *Phytophthora* as other fungi, *Pythium* spp. and various Mucorales, will also cause lesions (Schmitthenner & Bhat, 1994). Leaves of evergreen plants such as rhododendron, camellia, *Ilex* sp. (holly) or *Lithocarpus densiflorus* can be used for baiting *Phytophthora* spp. (Hüberli *et al.*, 2013; Martin *et al.*, 2012). Leaves that are thick and leathery are more suitable for *Phytophthora* baiting than brittle leaves which are often colonised by a diverse range of organisms. Rhododendron leaves were found to yield a greater diversity of

Phytophthora spp. and populations (Martin *et al.*, 2012). In Australia, water surveillance for *Phytophthora* spp. was done using *Pittosporum undulatum*, *Quercus robur, Hakea laurina, Banksia attenuata, Eucalyptus* spp. leaves and germinated lupin, *Lupinus angustifolius,* seedlings (Hüberli *et al.*, 2013). Most *Phytophthora* spp. were isolated from *Pt. undulatum* and *B. attenuata* and this is because they did not degrade while the lupin seedlings and *Q. robur* leaves often decomposed (Hüberli *et al.*, 2013). Leaves of *Eucalyptus* spp. were not effective for baiting in waterways with infrequent isolation of *Phytophthora* (Hüberli *et al.*, 2013). Water surveillance in six sub-catchments in the Waitakere Ranges in Auckland showed lupin seedlings, Himalayan cedar (*Cedrus deodara*), and kauri leaves resulting in poor isolation of *Phytophthora* spp. with most isolates recovered from rhododendron and kohuhu (*Pittosporum tenuifolium*) leaves (Randall, 2011).

1.2.3 Isolation of *Phytophthora* from soil

Soil baiting, and plating methods have also been successful in *Phytophthora* isolation (Ivors, 2018; Martin *et al.*, 2012). The soil plating method is used when there are high concentrations of *Phytophthora* inoculum in soil. This method uses only small amounts of soil that are placed on the surface of selective agar and is not suitable for recovery of *Phytophthora* spp. that are present only as a few propagules per gram of soil e.g. *Ph. cinnamomi* (Martin *et al.*, 2012). In the soil baiting method, a larger volume of soil is tested which increases the opportunity of isolating *Phytophthora* spp. that are at a low density (Martin *et al.*, 2012). Apart from this, the dormant spores of homothallic species are more likely to be detected by the baiting method than by plating (Martin *et al.*, 2012). Direct isolation of *Phytophthora* by plating is difficult because there is a high chance of *Pythium* growth on the selective media which will often outgrow the *Phytophthora* colonies and only experts are able to distinguish young *Phytophthora* from *Pythium* colonies using mycelial characteristics (Schmitthenner & Bhat, 1994). Soil sample should be taken from moist soil around healthy roots at the edge or drip line of the plant canopy where root growth is more vigorous or after wet weather which increases *Phytophthora* activity in soil (Drenth & Sendall, 2001).

In soil baiting methods, air dried soil is flooded with distilled water using a water to soil ratio of 2 : 1 (volume) and unwounded baits are floated in the water (Martin *et al.*, 2012). Zoospores swim upwards and colonise the floating bait (Ivors, 2018; Martin *et al.*, 2012). After five to seven days incubation at 21°C to 23°C (Meszka & Michalecka, 2016) the baits are collected, washed using distilled or tap water to avoid bacteria build up on the tissue surface and blotted dry prior to inoculating the discoloured tissue sections (5 mm diameter segments cut from individual necrotic spot) on selective media for *Phytophthora* isolations (Martin *et al.*, 2012).

1.2.4 Isolation of *Phytophthora* from plant material

Phytophthora spp. when associated with a diseased plant, are likely to be the causal agent of the disease as most species attack only living or freshly wounded tissue (Drenth & Sendall, 2001).

Phytophthora spp. are commonly primary invaders and do not invade plant tissue that has been invaded by other microorganisms and therefore are not regarded as secondary colonisers of diseased tissue (Drenth & Sendall, 2001). Isolation of *Phytophthora* from plant tissue is generally simple and successful if the tissue is fresh, while it becomes more difficult to isolate from necrotic tissues (Drenth & Sendall, 2001). This is because *Phytophthora* have poor saprophytic capabilities and very few mycelia remain once the host tissue dies (Drenth & Sendall, 2001). Additionally, chlamydospores and oospores germinate and emerge slowly from senescent plant tissue (Drenth & Sendall, 2001). *Phytophthora* spp. can be present on/in healthy plant tissue with no obvious symptoms and necrotic tissue tend to have many secondary pathogens which makes isolation of *Phytophthora* difficult from dead plants (Drenth & Sendall, 2001). Therefore, baiting and isolation is effective when the sample is taken from slightly diseased trees or when soil sampling is done around dead trees for baiting (Drenth & Sendall, 2001).

Successful isolation of *Phytophthora* spp. from tissue can be done from freshly infected tissue and it is important to obtain the sample from the edge of an actively growing lesion containing both diseased and healthy tissue (Drenth & Sendall, 2001). Surface sterilized tissue is transferred onto selective media and regular inspection of plates are done for emergence of hyphae (Drenth & Sendall, 2001). *Pythium* spp. are found on both healthy and diseased plant roots and crown which can affect *Phytophthora* growth in cultures. This can be avoided by surface sterilization of samples; choosing other plant tissue for culturing as *Pythium* is often confined to roots and badly rotted lower parts of the stem, or use of the centre part of the root for isolation and adding hymexazol to the media (Drenth & Sendall, 2001).

1.2.5 Culture media for *Phytophthora* spp. isolation

The choice of growth medium should be kept consistent for species description and identification (Martin *et al.*, 2012). PARPH is a *Phytophthora* selective medium based on cornmeal agar (CMA) that contains antibiotic and antifungal amendments such as pimaricin, ampicillin, rifampicin, pentachloronitrobenzene (PCNB), and hymexazol (Ferguson & Jeffers, 1999). Commonly used media include cV8 (clarified vegetable juice agar) which is used for studying growth and temperature relationship and morphology of different *Phytophthora* spp., while CMA and potato agar dextrose agar (PDA) are also used to study colony patterns (Martin *et al.*, 2012), as colony characteristics can be used to make preliminary identification to species level (Jeffers, 2006).

Cornmeal agar is not a nutrient rich media and is very suitable for isolation of *Phytophthora* spp. (Drenth & Sendall, 2001). PARPH-V8 agar was found to be as effective as the standard PARPH medium (with cornmeal basal medium) for isolation and recovery of *Phytophthora* spp. however, the colony morphology differ on PARPH- V8 (Ferguson & Jeffers, 1999). An advantage of using PARPH-V8 is that homothallic *Phytophthora* spp. sporulate and form sporangia more readily on V8 juice based medium than on cornmeal medium while only mycelium is usually produced on PARPH

medium (Ferguson & Jeffers, 1999). This enables distinguishing of certain species directly from isolation plates (Ferguson & Jeffers, 1999).

Cornmeal agar and V8 agar that is amended with a combination of antibiotic such as pimaricin (P), ampicillin (A), rifampicin (R), nystatin and fungicides such as pentachloronitrobenzene (P) and hymexazol (H) can be used to isolate *Phytophthora* spp. from necrotic sections of the leaf (Martin *et al.*, 2012; Stamler *et al.*, 2016b). The selectivity of the media is mainly due to nystatin and pimaricin which are active against Eumycota fungi and these media are light sensitive and therefore should be stored in the dark (Martin *et al.*, 2012). Pimaricin concentration of 5 (P_5) to 10 (P_{10}) ppm is more suitable for isolation of *Phytophthora* from old plant tissues (Drenth & Sendall, 2001). Isolation of *Phytophthora* spp. is often prevented by contamination by *Pythium* spp., however the addition of hymexazol can be used to suppress *Pythium* (Randall, 2011). Hymexazol inhibits most *Pythium* and *Morteriella* spp. that overgrow *Phytophthora* colonies (Martin *et al.*, 2012) however, although several *Pythium* spp. such as *Py. irregulare* and *Py. vexans* are resistant (Drenth & Sendall, 2001), many *Phytophthora* spp. are sensitive to hymexazol (Martin *et al.*, 2012) such as *Ph. cactorum, Ph. palmivora, Ph. lateralis, Ph. cinnamomi* and *Ph. citrophthora* while *Ph. infestans* is completely intolerant (Drenth & Sendall, 2001; Jeffers & Martin 1986). Hymexazol added at a final concentration of 25-50 µg/mL in the P_5ARPH media has been recommended by Drenth and Sendall (2001) for effective *Phytophthora* isolation. Therefore, the use of isolation media with and without hymexazol is recommended to target the isolation of a wide range of *Phytophthora* spp. from samples.

1.2.6 Culture independent methods for detecting *Phytophthora* spp. communities

Molecular techniques are increasingly been used to determine microbial communities in environmental samples and have the advantage of being culture-independent. Genetic fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) are commonly used for studying the diversity of microbial communities and has been used to identify *Phytophthora* species in forestry samples (Rytkönen *et al.*, 2012). However, the authors stated that closely related species could not be distinguished by the method. A new technique for *Phytophthora* detection has been developed which depends on obtaining DNA from environmental samples (plant material, soil and water) and metabarcoding using high-throughput sequence (HTS) (Burgess *et al.*, 2016; Redondo, 2018). This technique allows rapid characterization of microbial communities without isolation of the target organism on culture media (Català *et al.*, 2017). This technique is reported to be a more efficient method for *Phytophthora* community studies as it detects three time more *Phytophthora* spp. than the traditional methods (baiting of *Phytophthora* using plant baits) (Català *et al.*, 2017; Redondo, 2018). However, identification of the species within samples depends on the species coverage in the databases used, and as such any new species will not be able to be identified using this method. Further, if cultures are required for identification of new species, or additional studies on their

pathogenicity or biology parallel recovery of *Phytophthora* isolates from the samples will need to be conducted.

1.3 Intraspecific hybridization between *Phytophthora* spp.

Hybridization occurs readily between two allopatric species that have not previously co-existed, and have permissive reproductive barriers that allows the occurrence of interspecific mating (Stukenbrock, 2015). Hybridization is an important mechanism that could give rise to new plant pathogens (Stukenbrock, 2015). In the past decade reports of intraspecific hybridization in *Phytophthora* has become more common and this provides the pathogen with genomic plasticity (Burgess, 2015). During sympatric speciation (new species evolving from a single ancestor), reproductive incompatibility amongst species exists to maintain species integrity and avoid species fusion, however similar selection pressure do not exists with allopatric species (Burgess, 2015). The rise in hybridization can be due to anthropogenic activities that unite allopatric species (Burgess, 2015).

Hybrids tend to have a wider host range than their parental species, are more aggressive and capable of becoming dominant in the region (Ersek & Nagy, 2008). Several hybrids exist in the genus *Phytophthora* that are important plant pathogens. The notorious pathogen *Ph. alni* from clade 7, that is initially believed to have formed in nurseries through the hybridization between *Ph. uniformis* and *Ph.* × *multiformis* and later introduced into the natural environment, causes disease in alder trees in Europe (Burgess, 2015). A well-known hybrid *Ph. x serendipita* formed in clade 1 between *Ph. cactorum* and *Ph. hedraiandra* causes disease in horticultural crops and in nurseries (Burgess, 2015). Clade 1 hybrid *Ph. andina,* formed between *Ph. infestans* and another unknown but related species, is pathogenic to wild and cultivated *Solanum* species (Burgess, 2015). Three hybrids have also been described recently from clade 8b that affect winter vegetables and these hybrids have a different host preference than their parental species (Burgess, 2015). Several hybrids exists in clade 6 that have been isolated from natural waterways, with parental species such as *Ph. chlamydospora*, *P. amnicola* and *P. thermophila* (Burgess, 2015). High numbers of hybrids were collected from Western Australia waterways with up to six different parent species involved in 30 potential hybrid combinations (Burgess, 2015). *Phytophthora moyootj* was found to be associated with several hybrids that, based on DNA studies, consisted of combinations of up to three species. Many of these hybrids however were found to be sterile in culture which made them difficult to store long term to allow further study (Burgess, 2015). The reason why these hybrids are sterile under laboratory conditions could be due to meiotic incompatibility resulting in oospores that are abortive, unviable or deformed and have poor development of germ tubes (Burgess, 2015). Hybrids formed through sexual reproduction which are fertile in their natural environment are capable of back crossing that could lead to fusion of species or form new species overtime (Burgess, 2015).

1.4 Morphological identification of *Phytophthora*

The genus *Phytophthora,* belongs to the kingdom Chromista or Stramenopila (Agrios, 2005), phylum Oomycota and the family Pythiaceae (Lamour, 2013; Redondo, 2018). The genus *Phytophthora* was firmly established by 1925 with its features described by Tucker in a monograph published in 1931, with Waterhouse (1963) reviewing these descriptions in 1931, which resulted in the sorting out of synonyms and the addition of a key based on the morphological features of structures that brought more order to the genus (Lamour, 2013). The *Phytophthora* identification system developed led to the subdivision of the genus into six groups (I-VI; Table 1.1) (Thines, 2013).

Table 1.1: Six group classification of *Phytophthora* by Waterhouse, source Lamour (2013).

The major review of the genus done by Waterhouse in 1963 remained the basis for *Phytophthora* identification and taxonomy (Drenth & Sendall, 2001), which was later revised and adjusted (Brouwer *et al.*, 2012). However, identification of *Phytophthora* spp. using morphology has many drawbacks (Thines, 2013). Difficulties exist in accurately identifying some species as there are few or variable difference amongst some species (Drenth & Sendall, 2001). *Phytophthora* is known as a taxonomically difficult genus because many cultures used in species identification are plastic i.e., variation within the population can occur (e.g. sporangial size or sporangial or oogonium morphology), have overlaps between species (overlap of spore sizes in different taxa), are greatly influenced by environment (morphology of structures varies under different conditions) with unknown genetic basis (Drenth & Sendall, 2001). Therefore, morphological identification is used together with molecular methods in identifying the *Phytophthora* spp.

For identification of *Phytophthora* spp. cultures should be done using either the hyphal tip, a single germinated zoospores cyst, oospore or a sporangium (Drenth & Sendall, 2001). For species identification it is important to induce production of asexual (zoospores, sporangia, chlamydospores) and sexual structures (oospores), however on selective media most *Phytophthora* spp. will not sporulate and form characteristic propagules (Drenth & Sendall, 2001). To overcome this problem Drenth and Sendall (2001) recommends incubation of suspected cultures in optimum growing temperatures on a natural medium such as lima bean agar, V8 juice or carrot agar. Colony types

formed on different growing medium vary and mycelium growth habit such as aerial or appressed and mycelium pattern (Figure 1.1) is also used in identification of the cultures, however consistency should be maintained in the type of media used to standardize the observations (Jeffers, 2006).

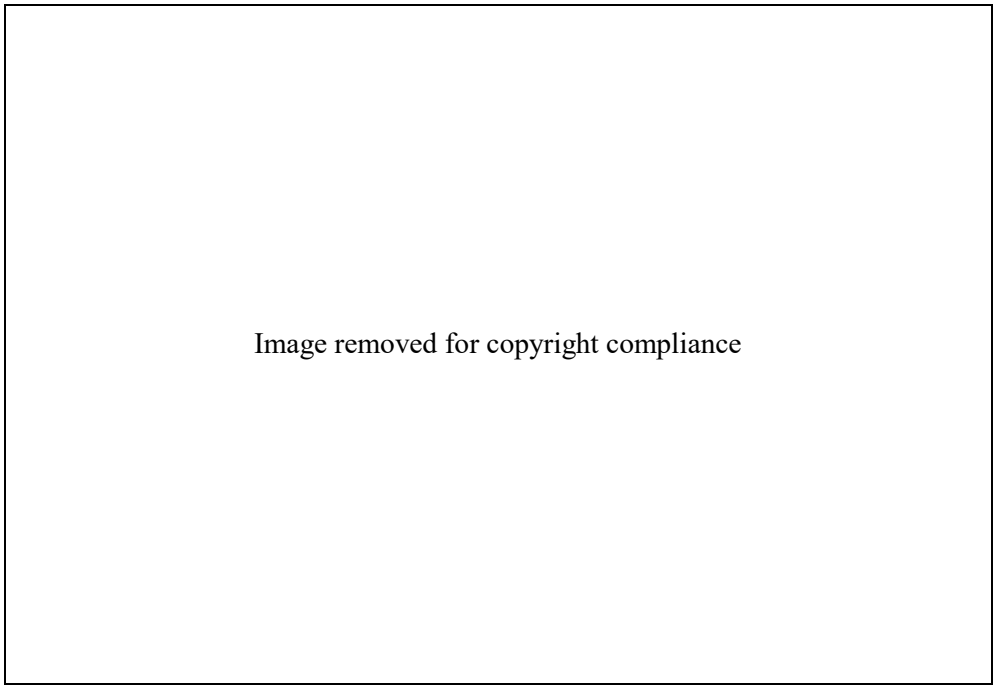

Figure 1.1: Mycelium morphology. (A) Uniform mycelium, (B) and (C) coralloid and knobby mycelium, and (D) coiled mycelium. Source Gallegly and Hong (2008a).

Morphological studies in *Phytophthora* identification look at characteristics such as sporangial papillation (Figure 1.2), attachment of the antheridia (Figure 1.3), shape of the sporangia (Figure 1.4), morphology of the sporangiophore (Figure 1.5) and caducity (shedding of sporangium at maturity), presence of hyphal swellings and chlamydospores (Figure 1.6), heterothallism or homothallism in sexual reproduction (Drenth & Sendall, 2001), oogonium (female gametangia) (Figure 1.7) and oospore (Figure 1.8) characteristics (Jeffers, 2006). Antheridia can be separated into two types, being amphigynous (the antheridium surrounds the oogonial stalk) and paragynous (the antheridium does not surround and can be attached anywhere on the oogonium close to the oogonium stalk) however, many *Phytophthora* spp. can produce both types of antheridia (Q-bank, 2017). Antheridia are also classified based on the hyphal bearing i.e., monolinous antheridia where the hyphae bearing the antheridium branches from the same hyphae as the oogonium or it can be produced on a different hyphae in case of diclinous antheridia (Q-bank, 2017).

For most morphological keys of *Phytophthora* the different species are initially divided based on the sexuality pattern (Gallegly & Hong, 2008a). *Phytophthora* spp. are bisexual (produce male and female gametangia) with only half the species being homothallic that rapidly and abundantly produces oospores in a single culture (Drenth & Sendall, 2001). The remaining *Phytophthora* spp. are

heterothallic and only produce gametangia due to chemical stimulation from the opposite mating type (Drenth & Sendall, 2001). Homothallism allows self-fertilization while heterothallism encourages outbreeding (Drenth & Sendall, 2001) and some fresh cultures of heterothallic species can also be self-fertile in a single culture (Martin *et al.*, 2012).

Homothallic species are separated based on the type of antheridium (Gallegly & Hong, 2008a). Asexual characters, such as type of papillae, length of pedicle and caducity are used within the sexual group (Gallegly & Hong, 2008a). Ability to grow at 35°C is used for separating some of the heterothallic species (Gallegly & Hong, 2008a). Maximum temperature at which a colony can grow is used for identification of isolates and is an important part of the morphological key (Gallegly & Hong, 2008a). Therefore unknown isolates should be grown at 20°C, 25°C, 30°C and 35°C for identification (Gallegly & Hong, 2008a).

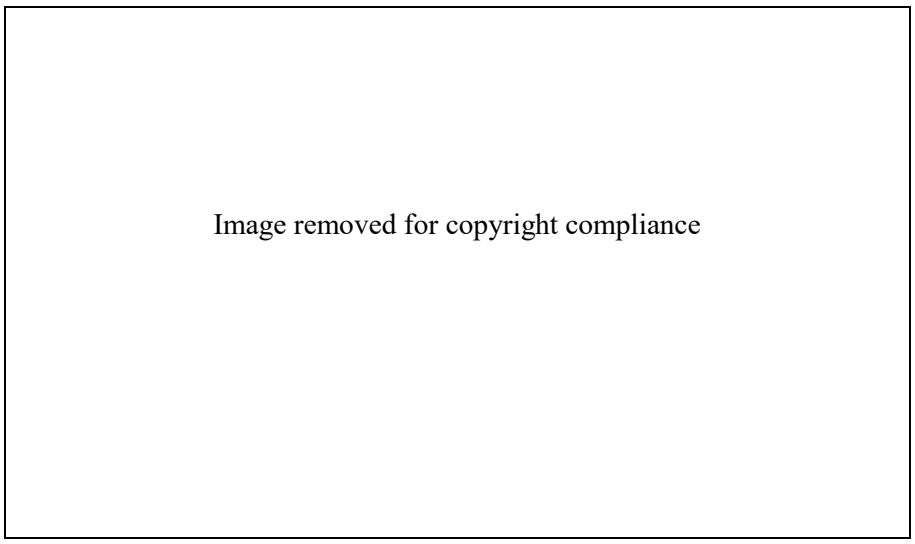

Figure 1.2: Three different types of sporangial papillation in *Phytophthora*, Source "Forest Phytopthoras of the world" 2017).

Figure 1.3: Two types of antheridial attachment, A) paragynous attachment, and B) amphigynous. Source Drenth and Sendall (2001).

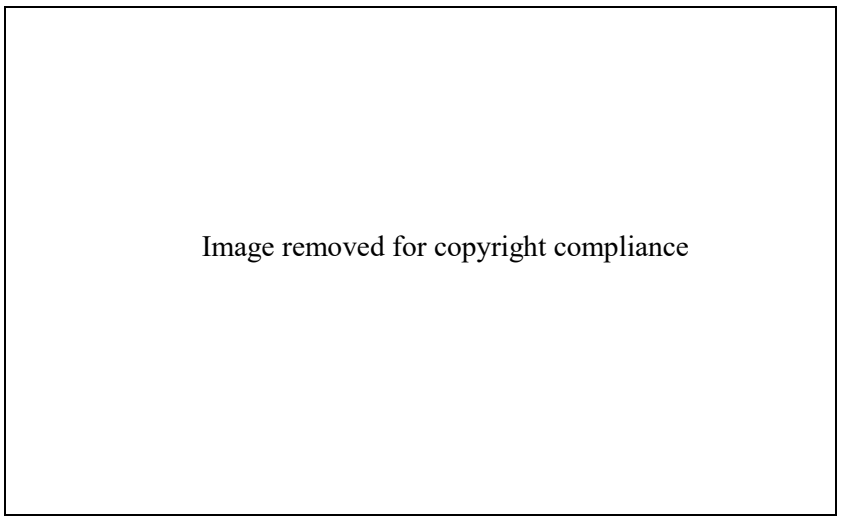

Figure 1.4: Different types of sporangium shapes. Source Drenth and Sendall (2001).

Figure 1.5: Different types of sporangiophore morphology, Source Drenth and Sendall (2001).

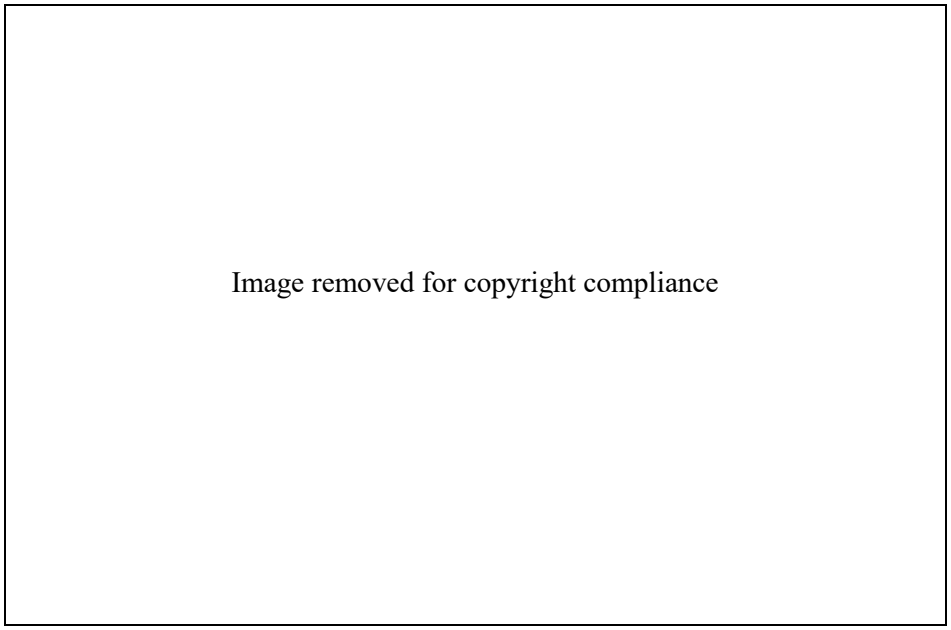

Figure 1.6:Chlamydospore morphology. (A) terminal chlamydospore, (B) intercalary chlamydospore, (C) lateral chlamydospore, and (D) clustered chlamydospores. Source Gallegly and Hong (2008a).

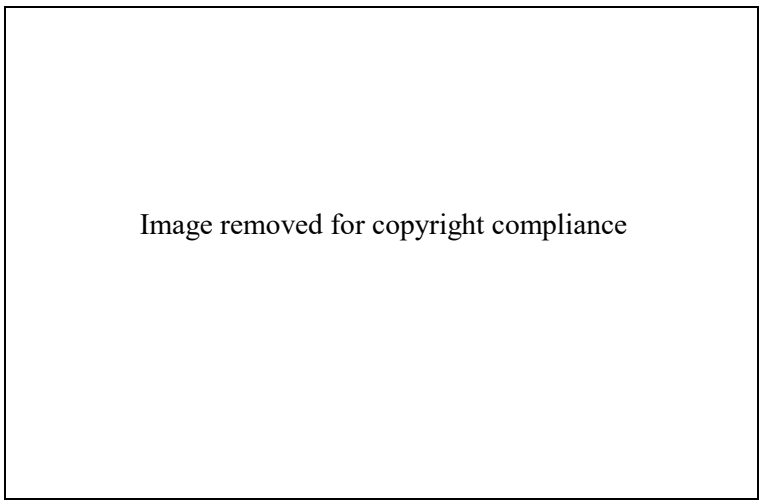

Figure 1.7: Characteristics of oogonia. (A) smooth globose oogonium, (B) ornamented oogonium, (C) oogonium with wavy wall, (D) hooked oogonium, (E) oogonium with a tapered base within an antheridium, (F) oogonia with a tapered base above the antheridium, (F) oogonia with a tapered base above the antheridium, (G) pigmented oogonium. Source Gallegly and Hong (2008a).

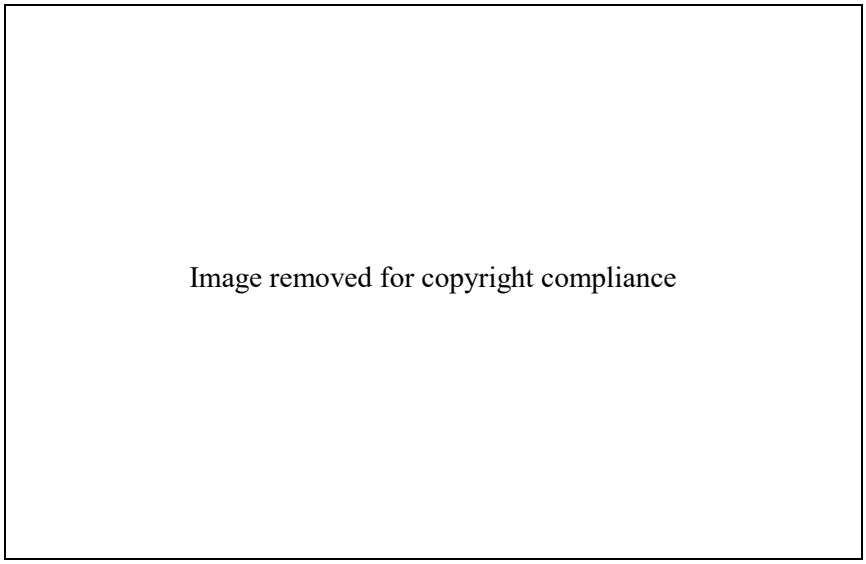

Figure 1.8: Oospore morphology. (A) plerotic oospore, (B) aplerotic oospore, (C) thick-walled oospore, and (D) thin-walled oospore. Source Gallegly and Hong (2008a).

1.5 Difference between *Phytophthora* and *Pythium*

The genera *Phytophthora* and *Pythium* both belong to the family Pythiaceae and so are very closely related. One of the main distinguishing features between these two genera are the way zoospores are produced; but also there are differences in the sporangia and antheridia (Drenth & Sendall, 2001). *Phytophthora* spp. zoospores are produced in the sporangium while in *Pythium* spp., zoospores develop inside a vesicle that is produced by the sporangium (Figure 1.9) (Drenth & Sendall, 2001). *Phytophthora* spp. sporangia are always terminal and usually obpyriform or ovoid in shape, while *Pythium* sporangia can be globulose, filamentous, lobate and frequently intercalary (Drenth & Sendall,

2001). *Pythium* spp. have paragynous antheridia that can be attached at any point on the oogonium and many antheridia can be attached on a single oogonium (Drenth & Sendall, 2001). In *Phytophthora* spp. the antheridium attaches onto the oogonium at the lower hemisphere (Drenth & Sendall, 2001).

Figure 1.9: Development of a vesicle from a sporangium in *Pythium* spp. containing zoospores, Source (Drenth & Sendall, 2001)

1.6 Molecular identification

Molecular techniques were adapted in the 1990s which led to a better understanding of *Phytophthora* taxonomy (Lamour, 2013). DNA based identification system are commonly used in combination with morphological data (Brouwer *et al.*, 2012). The Internal transcribed spacer (ITS) and rRNA DNA regions are the most common regions used for identification of oomycetes to species level. The ITS is non-encoding, evolves rapidly and is highly variable (Rahman *et al.*, 2014). The flanking genes of the ITS1 and ITS2 region contain highly homologous stretches that are used to design primers to be used in the polymerase chain reaction (PCR) and the same primers can be used for almost all *Phytophthora* species (Brouwer *et al.*, 2012). An advantage of the ITS approach is that these sequences can be easily obtained resulting in a high number of ITS sequence data in databases such as GenBank. However, in some cases there is a lack of variation between the ITS sequence of species that are closely related (Brouwer *et al.*, 2012). Due to this, the use of either mitochondrial or nuclear encoding proteins have been used in sequencing (Brouwer *et al.*, 2012). Other coding regions that are used in phylogenic studies of *Phytophthora* include the large subunit of the nuclear rRNA gene (nLSU) and genes encoding cytochrome c oxidase subunits I and II (*coxI* and *coxII*) (Rahman *et al.*, 2014) which is a mitochondrial gene (Brouwer *et al.*, 2012). In some cases, the *coxI* gene has been found to be more discriminative than the ITS region and so using both these sequence is recommended in the identification of the *Phytophthora* spp. (Rahman *et al.*, 2014). Kroon et al. (2004) conducted the first overall phylogenetic analysis of *Phytophthora* using mitochondrial and multiple nuclear genes which revealed discrepancies in the phylogenetic position of a number of *Phytophthora* species within the taxonomy indicating interspecific mating or somatic hybridization amongst species (Brouwer *et al.*, 2012).

Studying interspecific hybrids using the ITS region is not useful as the ITS1 and ITS2 are highly variable and non-coding which results in unreadable sequences (Burgess, 2015). Other gene regions such as the β-tubulin and Heat shock protein 90 (HSP) can be used in molecular studies along with the anti-silencing factor (ASF) gene found to be the easiest to amplify for clade 6 species (Burgess, 2015). A wider multi-gene phylogeny study carried out by Blair et al. (2008) using sequences for seven nuclear genes defined the current 10 clades and subclades of *Phytophthora,* which is outlined in Section 1.7.

1.7 Ten clades and subclades of *Phytophthora*

Clade 1 which is a well-studied group of *Phytophthora* species comprising 13 species including *P. infestans* (Brouwer *et al.*, 2012). Three new species have been identified and added to this clade since 1996 and these include *Ph. andina, Ph. ipomoeae* and *Ph. hedraiandra* (Brouwer *et al.*, 2012). *Ph. andina* and *Ph. ipomoeae* are closely related to *Ph. infestans*, while *Ph. hedraiandra* forms hybrids with *Ph. mirabilis* (Brouwer *et al.*, 2012). *Phytophthora* spp. in clade 1 are papillate or semi-papillate (with only one type present in a subclade) (Brouwer *et al.*, 2012). Subclade 1a and 1b have papillate zoosporangia with paragynous attachment of antheridia and oogonia (Brouwer *et al.*, 2012), with species in subclade 1a and 1b mainly infecting roots (Brouwer *et al.*, 2012). Subclade 1c have semi-papillate zoosporangia developing on sporangiophores that are developed distinctively and deciduous sporangia that are spread through aerial dispersal (Brouwer *et al.*, 2012). *Ph. nicotianae* has not been put in any of the three subclades based on the sequence analysis and is the only species placed on its own (Brouwer *et al.*, 2012). *Ph. nicotianae* has papillate sporangia and amphigynous antheridia (Brouwer *et al.*, 2012).

Clade 2 is one of the largest clades with 14 new species added since 1996 making a total of 21 species in the clade (Brouwer *et al.*, 2012). All species in this clade have either papillate or semi-papillate zoosporangia with 15 species having homothallic mating system (Brouwer *et al.*, 2012). Species in this clade are soil-borne pathogens that infect roots causing decline and diebacks in forests and nurseries (Brouwer *et al.*, 2012). Based on the DNA sequencing of isolates from a soil-borne *Phytophthora* spp. survey, *Ph. plurivora* was identified as one of the most common pathogens causing decline in forests in Europe (Brouwer *et al.*, 2012). *Ph. bisheria* is a new species that has been found to cause root rot in strawberries (in the USA), root rot in roses (the Netherlands) and root rot in raspberries (Australia) (Brouwer *et al.*, 2012). New species such as *Ph. multivora* and *Ph. elongate,* which are homothallic, and *Ph. frigida* which is heterothallic and papillate, occur in South Africa causing root rot in eucalyptus trees (Brouwer *et al.*, 2012).

Initially **clade 3** had only one species, *Ph. ilicis,* with three new species added in the last decade (Brouwer *et al.*, 2012). Species in this clade have semi-papillate sporangia, are homothallic and affect trees (Brouwer *et al.*, 2012). *Ph. pseudosyringae* causes decline in oaks and affects fine roots and

stems of beech in Europe (Brouwer *et al.*, 2012). *Phytophthora nemorosa* causes myrtle wood canker, while *Ph. psychrophila* has been isolated from oak but has not been found to cause decline (Brouwer *et al.*, 2012).

Clade 4 is another small clade that has expanded after 1996 with currently seven *Phytophthora* spp. (Brouwer *et al.*, 2012). These species are mainly pathogen on roots, have papillate sporangia and be either homothallic or heterothallic (Brouwer *et al.*, 2012). Other species in this clade include *Ph. quercetorum* that is frequently isolated from soil in the USA and on *Quercus* sp. in Europe; *Ph. alticola* was found on *Eucalyptus* and *Ph. arenaria* causes dieback in Western Australia (Brouwer *et al.*, 2012).

Clade 5 is the smallest clade with only four species,, *Ph. heveae, Ph. katsurae* (Brouwer *et al.*, 2012) and two recently identified species *Ph. agathidicidia* and *Ph. cocois (Weir et al., 2015).* These species have papillate sporangia and are homothallic (Brouwer *et al.*, 2012). Apart from this, they have amphigynous antheridia with *Ph. katsurae* having distinct ornamented oogonia (Brouwer *et al.*, 2012).

Clade 6 has expanded significantly since 1996 with the addition of 20 new species making a total of 23 species in this clade (Brouwer *et al.*, 2012). These 20 species have been identified from waterways around the world indicating a saprophytic phase in their lifestyle (Hüberli *et al.*, 2013). Most clade 6 pathogens infect roots or are found in the rhizosphere, with a few exceptions such as *Ph. pinifolia* that attacks foliar plant parts (Brouwer *et al.*, 2012). Most species are non-papillate with only two species having semi-papillate sporangia (Brouwer *et al.*, 2012). Twelve out of the 23 species are either fully or partially sterile sexually with one species being heterothallic (Brouwer *et al.*, 2012). Many undescribed isolates in clade 6 were identified after a large-scale survey of *Phytophthora* spp. in Western Australia waterways that resulted in identification of five new species (Brouwer *et al.*, 2012).

Six out of the 13 species in **clade 7** were described prior to 1996 and all the species are pathogenic on roots (Brouwer *et al.*, 2012). These species have non-papillate sporangia with random distribution of heterothallism and homothallism in subclade 7b, while subclade 7a contain more homothallic species (Brouwer *et al.*, 2012). This clade contains *Ph. cinnamomi* which is a destructive pathogen in native forests of Australia and affect horticulture in many parts of the world, including New Zealand.

Clade 8 together with clades 2 and 6 make up the three largest clades in the genus *Phytophthora*. Since 1996 seven new species have been added to clade 8 which is separated into a total of four subclades. Of the 18 species in this clade 15 are homothallic (Brouwer *et al.*, 2012). *Phytophthora ramorum* is one of the most destructive pathogens in this clade responsible for causing Sudden Oak Death (Brouwer *et al.*, 2012). All species in the subclade 8a are non-papillate while the other three subclades are semi-papillate (Brouwer *et al.*, 2012).

Clade 9 is one of the less-resolved clades with numerous new species identified after 1996 (Brouwer *et al.*, 2012). This clade contains pathogens such as *Ph. parsiana* which causes disease in almonds, fig and pistachio; *Ph. irrigata* isolated from irrigation water causing disease on Azalea plants and *Ph.*

fallax and *Ph. captiosa* which cause crown rot in eucalyptus in New Zealand (Brouwer *et al.*, 2012). Except for *Ph. macrochlamydospora, s*pecies in this clade are mostly found in soil, are non-papillate and homothallic (Brouwer *et al.*, 2012).

Clade 10 contains only four species and except for *Ph. gallica* the other three species are papillate and attack foliage (Brouwer *et al.*, 2012). This clade contains pathogens such as *Ph. gallica* which causes decline in Oak in Germany, *Ph. kernoviae* a pathogen of beech in the United Kingdom, and *Ph. morindae,* a new species which causes fruit rot and foliar blight in noni (*Morinda citrifolia*) (Brouwer *et al.*, 2012).

1.8 Research context and objectives

In the last decade new *Phytophthora* spp. have been identified from forests and other ecosystems around the world, such as *Ph. ramorum* which has become an important pathogen of trees and woody ornamental plants (Huai *et al.*, 2013). *Phytophthora* spp. have been identified through broader surveys of soils and streams not associated with any observations of forest decline caused by *Phytophthora* (Huai *et al.*, 2013). Water surveys have become popular in regions where early detection of infected areas is important for containment and eradication of the *Phytophthora* spp. (Hüberli *et al.*, 2013). More than 20 new *Phytophthora* spp. such as *Ph. ramorum* have been identified from waterways around the world (Hüberli *et al.*, 2013). Research on *Phytophthora* spp. in New Zealand show the presence of 43 *Phytophthora* spp. however surveillance has been mainly done on soil and diseased plant samples (Lewis, 2018; Scott & Williams, 2014). Apart from a survey of waterways in the Waitakere Ranges, in the West Auckland for *Phytophthora* spp. (Randall, 2011), no other sampling of waterways has been carried out in New Zealand. Therefore, water surveillance can provide opportunities to sample and test a wider area draining into the waterways that will provide better information on the *Phytophthora* spp. present in the Canterbury region.

The objectives of this research, and the hypothesis to be tested are as follows:

1. Identification of the best method for isolating *Phytophthora,* including the plant bait, from waterways. The different methods, which includes direct baiting in waterways, water baiting in the laboratory and water filtration, will be tested in a preliminary study to determine the best method for *Phytophthora* isolation. The hypothesis tested was that different methods and plant baits will isolate different *Phytophthora* species and number of isolates.
2. To identify different *Phytophthora* spp. present in Canterbury waterways representing different land use types, including sites along the Ashburton river, the Selwyn river, the Liffey stream, the Halswell River, and streams around Banks Peninsula (Kaituna Valley and Prices Valley) and Lake Hood. The hypothesis tested was that surrounding land used type and abiotic water characteristics will influence the *Phytophthora* species communities recovered from the waterways.

3. To investigate the pathogenicity of the *Phytophthora* species isolated from waterways and identify a rapid pathogenicity screening assay. The hypothesis tested was that the *Phytophthora* species isolated were potential pathogens.

Chapter 2: Identification of the best method for isolating *Phytophthora* species from waterways in Canterbury

2.1 Introduction

The genus *Phytophthora* consists of 124 described spp. (Lamour, 2013) which are mostly plant pathogens. Spread worldwide, *Phytophthora* spp. have been known to cause devastation to agriculture, forestry and the natural ecosystem. Historically, late blight of potato disease, caused by *Ph. infestans,* was the main contributing factor to the Irish potato famine that occurred between 1845 and 1852 resulting in the death of millions of people (Gibbons, 2017). In recent years *Phytophthora* has become an important issue due to the devastation of native forests. In Australia, *Ph. cinnamomi* has severely impacted the native biodiversity by affecting 191 plant species including 39 that are endangered (Global invasive species database, 2018). While in California 70% of avocado orchards have been affected, impacting livelihoods and the local economy (Global invasive species database, 2018). Apart from this, Sudden Oak Death disease, caused by *Ph. ramorum,* affects over 100 plants species causing forest decline in the USA and UK. This directly impacts recreational, cultural and economic use of the forest resource (Grunwald *et al.*, 2012). *Phytophthora agathidicida* in New Zealand causes kauri dieback disease, which has negatively impacted the natural ecosystem (Bassett *et al.*, 2017). There are, however, many *Phytophthora* spp. present in New Zealand that remain unknown.

Large areas of land draining into nearby waterways can be effectively surveyed for identification of the different *Phytophthora* spp. present in an area. *Phytophthora* sp. isolated from waterways are mainly, but not limited to, clade 6 species. Of the more than 20 *Phytophthora* spp. identified from waterways around the world commonly isolated species from clade 6 include *Ph. amnicola, Ph. fluvialis, Ph. gonapodyides, Ph. inundata, Ph. litoralis,* and *Ph. thermophila* (Hüberli *et al.*, 2013; Hwang *et al.*, 2008). Apart from clade 6, *Phytophthora* spp. from other clades have also been recovered from waterways including important plant pathogenic species such as *Ph. cryptogea* (clade 8), *Ph. ramorum* (clade 8), *Ph. cinnamomi* (clade 7) and *Ph. multivora* (clade 2) (Hüberli *et al.*, 2013). Although *Phytophthora* spp. are generally referred to as plant pathogens, *Phytophthora* spp. in clade 6 (Section 1.3) are hypothesised to be saprophytes that require an aquatic environment for asexual life cycle and dispersal (Hüberli *et al.*, 2013). Sporangia, formed on debris and roots on the soil surface, are washed into water pools and rivers (Drenth & Guest, 2004) and contribute to the *Phytophthora* spp. isolated from waterways.

Soil plating and baiting, isolation from diseased plant tissue, water baiting, and filtering methods have been used in previous studies for isolation of *Phytophthora* sp. The soil baiting and plating method is successful in isolating *Phytophthora* when there are high concentrations of *Phytophthora* inoculum in soil, however there can be issues with high levels of *Pythium* contamination (Martin *et al.*, 2012). Isolation of *Phytophthora* from plant tissue is generally simple and successful if the tissue is fresh,

while it becomes more difficult to isolate from necrotic tissues (Drenth & Sendall, 2001) since *Phytophthora* have poor saprophytic capabilities and very few mycelia remain once the host tissue dies (Drenth & Sendall, 2001). *Phytophthora* spp. isolation from waterways can be done by baiting or filtering methods (Martin *et al.*, 2012). For the baiting method, plant material generally plant leaf baits or seedlings are floated in water with isolations subsequently carried out from lesions caused by zoospores present in the water (Hüberli *et al.*, 2013; Randall, 2011). *Phytophthora* species isolates have also been recovered by filtering water samples through membrane filters to capture *Phytophthora* spores, which are then plated on selective media (Hwang *et al.*, 2008). Water surveys have become more widespread, especially in regions where early detection of infected areas is important for containment and eradication of *Phytophthora* spp. (Hüberli *et al.*, 2013).

The overall aim of the research presented in this chapter was to determine the most effective method for recovery of *Phytophthora* spp. from waterways. Three methods were evaluated i) river or *in situ* baiting, ii) laboratory baiting, and iii) filtration, with different plant tissue baits also evaluated for both the *in situ* and laboratory baiting methods. The hypothesis tested was that different methods and plant baits will isolate different *Phytophthora* species and number of isolates

2.2 Materials and Methods

A preliminary experiment was conducted to evaluate three baiting methods for recovery of *Phytophthora* species from waterways being i) river or *in situ* baiting with seven plant leaf baits, ii) water baiting in the laboratory with the same seven plant leaf baits, and iii) water filtration method. Two waterways around Lincoln (Liffey Stream and Halswell River) were sampled in summer, during February 2018. The best methods identified in this objective was used in a more intensive study in autumn during May 2018 (Objective 2).

2.2.1 *Phytophthora* baiting from waterways

Seven plant leaf baits (Appendix A.2.1) including *Rhododendron arboreum*, *Pittosporum undulatum, Banksia attenuata, Camellia japonica* (camellia), *Pittosporum eugenioides* (lemon wood), *Pinus radiata* (pine) and *Cedrus deodara* (Himalayan cedar) was used to bait for *Phytophthora* spp. from waterways in the preliminary baiting experiment. Branches of the bait were collected the day prior to baiting and placed in a vase with water at room temperature until used. Prior to baiting, the leaf baits were washed, dried and placed inside the bait bags. For pine and cedar, needle fascicles were used and for other leaf baits individual leaves were used.

2.2.1.1 River *(in situ)* baiting method

Bait bags (30 cm x 15 cm) made of insect mesh (Mitre 10) were sown with seven compartments each containing one of the seven leaf baits (Figure 2.1). The bait bags were attached with Styrofoam discs (5 cm diameter) to allow the bait to float just below the water surface and pebbles, placed in the

corners of the bags, were used to prevent the bags from floating on the water surface. Nylon wire was used to tie the bags to the river bank with a number tag for identification purposes. A notice on the purpose of placing the bait bags was also attached for public information. The GPS co-ordinates of each waterway sampling site was recorded using a hand-held GPS (Garmin GPSMAP 64). Three replicate bait bags, each containing the seven plant leaf baits, was placed in each of the waterway sampling sites. At each waterway sampling site, each bag was placed in different parts of the river (i.e., near the banks, in the middle and areas with fast water flows) with the aim to assess the optimum area for infection. However, it was difficult to maintain the baits in one place due to the strong water currents. The bait bags were retrieved after 7 days (Martin *et al.*, 2012), placed in an insulated cool box and taken back to the Lincoln University laboratory for processing (Section 2.2.2). From each baiting site, three 1 L water samples were collected using the Mighty Gripper (280 cm long three stage telescopic handled gripper; The Mighty Gripper Company Ltd) in clean plastic bottles to represent the three-sampling site where the *in situ* baits were placed (Figure 2.2). Around 300 mL of water was collected at three times for each sample, allowing a gap of 5-10 min of water flow between each sample collection. The temperature of the water immediately after collection was determined using a thermometer placed in the water of one container. The water samples were placed in a cool box and taken back to the Lincoln University laboratory and used for setting up the laboratory baiting and filtration experiments on the same day.

Figure 2.1: Bait bags made of insect mesh material divided into seven sections, each containing one leaf of the seven plant bait species.

Figure 2.2: The Mighty Gripper equipment used for collecting water samples within the waterway areas.

2.2.1.2 Water baiting in the laboratory

The water samples from each site collected during the river baiting (Section 2.2.1.1) was mixed to account for any variability in the *Phytophthora* spp. populations between samples. A 300 mL aliquot from each of the three 1 L samples was placed in a small plastic container (20 cm x 12 cm x 5 cm; Figure 2.3) in the laboratory and the plant leaf baits outlined in Section 2.2.1 floated for 7 days at room temperature (12°C to 22°C) on the laboratory bench. A layer of cling film was placed loosely on top of the containers to avoid loss of water from evaporation. One replicate for each of the seven plant tissue baits was placed in each replicate container for each site. After 7 days, the baits were collected and isolations from the lesioned tissue were carried out as outlined in Section 2.2.2.

Figure 2.3: Water baiting set up in the laboratory; (A) initial experimental set up, and (B) baits after 7 days with red arrows indicating typical discoloured or lesion tissue.

2.2.2 Culturing and isolation from baits

After collection, leaf baits were washed in tap water and placed in labelled plastic bags and stored at 8°C in the dark until processed. Isolations from the baits were carried out within 2-3 days of collection. Prior to culturing, the leaves were rinsed in tap water, surface sterilized using 10% bleach for 30 seconds (Ivors, 2015a), rinsed in sterile deionised water (Stamler *et al.*, 2016a) and dried on sterile tissue paper in a laminar flow hood. The plant leaf baits were then observed for any lesions or discoloured tissue and up to 10 (two pieces from each lesion) rectangular sections approximately 5-8 x 1-2 mm in size were cut from the margin of the lesions (Schmitthenner & Bhat, 1994). Five pieces were plated onto each of the two selective media (Appendix A.2.2) consisting of corn meal agar (CMA; Becton, Dickinson and Company ™) amended with 5 µg/mL pimaricin, 250 µg/mL ampicillin, 10 µg/mL rifampicin and 100 µg/mL pentachloronitrobenzene either without (P_5 ARP CMA) or with 50 µg/mL hymexazol (P_5 ARPH CMA) for isolation of *Phytophthora* spp. (Drenth & Sendall, 2001). The plates were incubated at 20°C in the dark and observed daily for colonies characteristic of *Phytophthora* spp. growing from the lesioned tissue. These were transferred onto 20% vegetable juice agar (V8A; Appendix A.2.2) and potato dextrose agar (PDA; Difco™) by cutting 5 mm^2 piece of agar from the edge of the colony (Safaiefarahani *et al.*, 2013) and placing one piece each on V8A and PDA. The plates were then incubated in darkness for two weeks at 20°C and observed daily for development of presumptive *Phytophthora* colonies.

2.2.3 Filtration method

Water samples were kept refrigerated (8°C) in the dark and processed within 10 hours of collection (Hwang *et al.*, 2008). Water samples, which contained large organic particles, were first filtered using cheese cloth. Prior to filtering, the water samples for each site were mixed together and two 100 mL subsamples per sample were vacuum-filtered (Figure 2.4) through membrane filters (3-µm pore size, 47 mm diameter: Whatman Nucleopore membranes) (Hwang *et al.*, 2008). The two filter papers from the 100 mL sub- samples were placed face down on either a P_5 ARP CMA plate or a P_5 ARPH CMA plate. The plates were then incubated for 3 days in darkness at 20°C after which the filter was aseptically removed from the plate (Martin *et al.*, 2012). The plates were incubated for a further 7 days and observed daily for colony growth. Colonies presumptively identified as *Phytophthora* spp. was sub-cultured onto fresh media as described in Section 2.2.2 (Hwang *et al.*, 2008).

The presumptive *Phytophthora* spp. cultures (Section 2.2.6) obtained from the three different isolation methods were sub-cultured onto PDA and V8A for morphological identification based on colony and spore characteristics. Colonies that grew faster than 15 mm per day were considered as *Pythium* or other oomycetes and were excluded from further analysis.

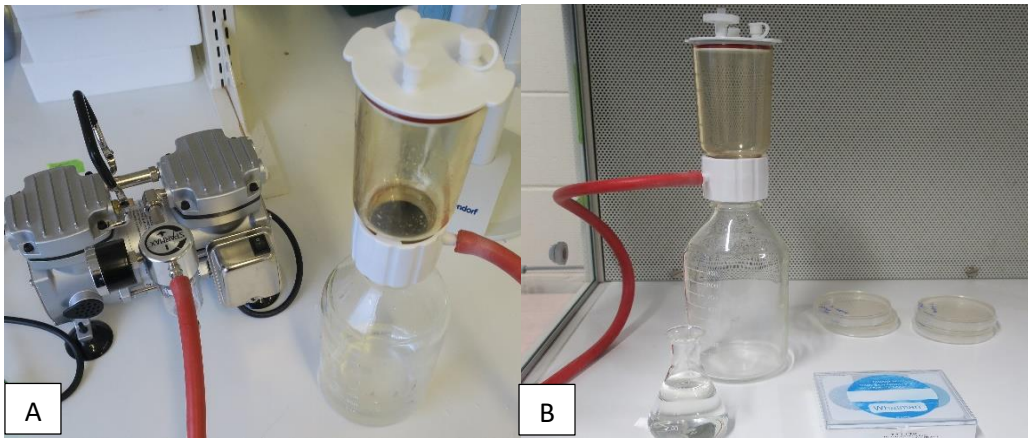

Figure 2.4: Vacuum pump used for water filtration; (A) top view and (B) side view of the set up inside the laminar flow.

2.2.4 Storage of isolates for molecular studies and pathogenicity test

Pathogenicity of *Phytophthora* cultures decrease after prolonged storage on media and cultures need to be transferred onto fresh media every two to four weeks to maintain vigour (Drenth & Sendall, 2001). Sterile water storage was used for long term storage of the cultures using the method described by Drenth and Sendall (2001). For each isolate, five small blocks (approx. 5 mm diameter) of actively growing culture on V8A were cut using a cork borer and placed in a small glass bottle with screw cap containing 25 mL of sterile distilled water (SDW). Caps were then tightened, and the bottles were stored at room temperature in the dark.

2.2.5 Morphological Identification

Morphological grouping of the *Phytophthora* isolates was done using colony growth pattern and growth rate on PDA. Colony growth rate was assessed based on the colony diameter, where fast growth indicated that the colony had almost reached the edge of the Petri plate in 7 days (7 cm to 9 cm colony diameter); medium growing colony had a diameter of 4.5 cm to 6.9 cm; slow growing colony grew 2.5 cm to 4.4 cm in diameter and a very slow growing colony had diameter between 0.1 cm to 2.4 cm after 7 days incubation. The isolates were further grouped based on the sporangia morphology according to the key provided in the forest Phytophthoras of the world website (http://forestphytophthoras.org/) and Gallegly and Hong (2008b).

Sporangia production was induced by growing *Phytophthora* sp. isolates on 20% V8A for 2 to 4 days, and agar plugs were cut from the colony edges and suspended in SDW in a Petri dish at room temperature (Drenth & Sendall, 2001) under natural day and night conditions for 3 days with sporangia production checked daily. Sporangia characteristics was observed under a microscope and recorded. For the *Phytophthora* sp. isolates that did not produce sporangia, distilled water was then replaced with 1% sterile soil extract solution (SESS; Appendix A.2.2) and the production of sporangia

observed after 24 hrs incubation under natural day and night conditions at room temperature. According to Drenth and Sendall (2001) some *Phytophthora* spp. such as *P. cinnamomi* do not produce sporangia in SESS and therefore the agar plugs for isolates that did not produce sporangia in SESS were placed in 1% nonsterile soil extract solution (NSSESS; Appendix A.2.2).

2.2.6 Identification by molecular techniques

For each morphotype (Section 2.3.2), 10-20% of isolates was identified to species level using the sequence of the ITS region of the rDNA (Hüberli *et al.*, 2013) with isolates from the different sample site selected. For morphotypes consisting of less than five isolates, all were sequenced. Sequencing of the Cytochrome c oxidase subunit 1 (*cox1*) gene region was used to confirm the identity of the isolates.

2.2.7 DNA extraction using Chelex solution

The DNA of each isolate was extracted by scrapping a small amount of mycelium from the edge of a 5-day old colony using a sterile pipette tip and placing into a microcentrifuge tube containing 200 µL of a 10% Chelex® 100 Resin solution. The mixture was vortexed for 10 sec and the tubes placed on a pre-heated heating block for 10 min at 100°C. The tubes were then removed from the heating block and vortexed followed by further heating at 100°C for 10 min, followed by centrifugation for 10 min at ≥13,000 rpm. The supernatant was then removed and placed in a clean microcentrifuge tube labelled with the isolate number. DNA quality and quantity were measured using a NanoDrop Spectrophotometer and the DNA was diluted with deionised water to concentrations of approximately 10 ng/µL. DNA was frozen at -20°C until used for PCR.

2.2.7.1 PCR amplification

Phytophthora genus specific primers for the internal transcribed spacer (ITS) region, 18ph2F and 28ph2R were used for initial identification (Table 2.1; Figure 2.5). Amplification of the DNA extract was done in a final volume of 20 µL which contained: 0.4 µL of each of the primers (final concentration 0.2 µM); 10 µL (1 unit) Dream *Taq* DNA polymerase (Thermo Scientific™) which includes Dream *Taq* DNA Polymerase, 2X Dream *Taq* Green buffer, dNTPs, and 4 mM $MgCl_2$ and 2 µL (approximately 20 ng) of DNA (Table 2.1). A negative control (no DNA) was also included in every PCR run. The PCR reactions were performed in a DNA Thermocycler and included one cycle of initial denaturation at 95°C for 3 min; 30 cycles of denaturation at 95°C for 20 secs, annealing at 61°C for 25 secs, and extension at 72°C for 1 min; a final extension at 72°C for 5 min (Appendix A.2.4; Table A1). The resulting product was stored at -20°C until used.

Partial sequences of the cytochrome c oxidase subunit I gene (*cox1*) was also generated to confirm the identity of selected isolates, including any isolates with low percentage identity and those potentially being hybrids based on sequencing of the ITS region. The *cox1* gene was amplified using primers FM84 and FM 77 (Table 2.1). The PCR reactions were performed with a final volume of 20 µL which contained; 0.4 µL of each of the primers (final concentration 0.2 µM), 10 µL (1 unit) Dream *Taq* DNA polymerase and 2 µL (approximately 20 ng) of DNA. A negative control (no DNA) was also included in every PCR run. The PCR cycle included one cycle of initial denaturation at 95°C for 4 min; 40 cycles consisting of denaturation at 95°C for 30 secs, annealing at 52°C for 45 secs, and extension at 72°C for 1 min; a final extension at 72°C for 7 min (Appendix A.2.4; Table A2). The resulting product was stored at -20°C until used.

Table 2.1: Details of the primer sequences used to amplify different target DNA regions used to identify *Phytophthora* isolates.

Target DNA	**Primer**	**Sequence**	**Expected length**	**Reference**
ITS (*Phytophthora* specific primer)	18ph2F	5'-GATAGACTGTTGCAATTTTGAGT-3'	1200 bp	Scibetta *et al.* (2012)
	28ph2R	5'- AAGGAACTTGCCCCAAGC-3'		
Cytochrome c oxidase subunit 1	FM84	5'-TTTAATTTTTAGTGCTTTTGC -3'	1299 bp	Martin and Tooley (2003)
	FM77	5'-CACCAATAAAGAATAACCAAAAATG -3'		

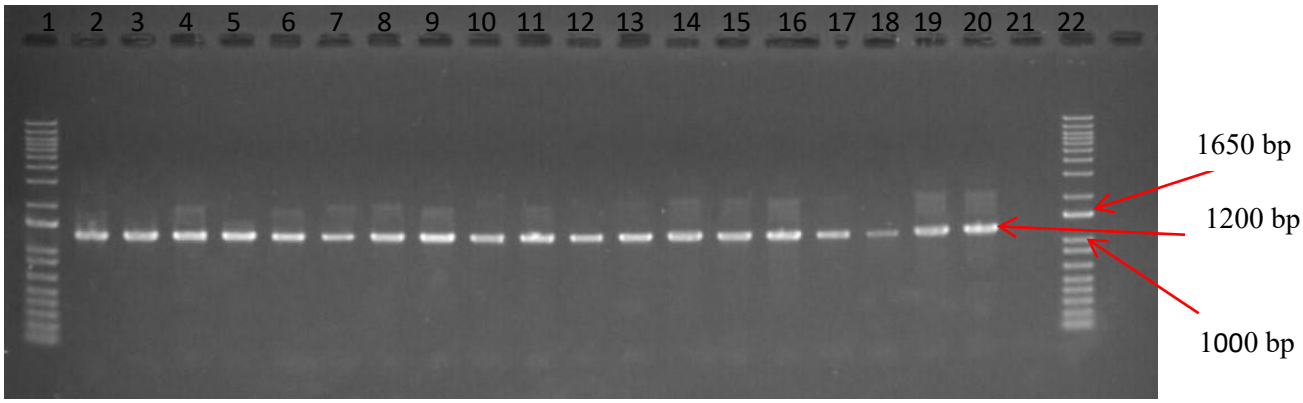

Figure 2.5: PCR products of the internal transcribed spacer (ITS) regions of isolates using Phytophthora specific primers (18ph2F and 28ph2R) separated on a 1% agarose gel. Amplification of *Phytophthora* spp. is indicated by a band at 1200 bp. Lanes: 1 and 22: 1 Kb Plus DNA ladder, 2-20: *Phytophthora* spp. band at 1200 bp, 21: negative control (no DNA).

The success in amplifying the product was checked by running a 10 µL aliquot of the PCR product in 1% agarose gel using 1 x Tris-Acetate-EDTA (TAE; Appendix A.2.2) buffer at 90 V for 40 min. A 1 Kb Plus DNA ladder™ (10 ng/µL) molecular weight marker was loaded in the first and last lane of each gel and a negative control (PCR product with no DNA) loaded in the last but one lane. The agarose gel was then stained in ethidium bromide (0.05 µL/mL) in a shaker for 15 min and then de-stained in water for 5 min. The stained gel was then photographed under UV light. A single bright band present for each sample was used to indicate successful amplification. The final PCR products were submitted to the Lincoln University Bio-Protection Research Centre for sequencing of the DNA using an ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems Foster City, California).

2.2.8 Analysis of sequences

The forward and reverse DNA sequences received from the Bio- Protection Research Centre were aligned using the BioEdit™ sequence alignment editor and manually edited for inconsistencies based on the chromatogram. The edited sequences were submitted to GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the basic local alignment search tool (BLAST) function to determine the *Phytophthora* sp. identification. Species identity was selected based on a reputable researcher, published source and sequence identity of 98% or above. Where more than one possible *Phytophthora* spp. was obtained (with 98% or 99% identity), evaluation was done on the prior known distribution of the spp. and its probability to occur in New Zealand.

2.2.9 Data analysis

All data analysis was performed in R (v 3.4.3) using the platform R Studio (v 1.1.419). Laboratory baiting and river baiting data from the Halswell River was analysed to identify the best baits and best *Phytophthora* recovery methods. The filtration method was not included due to lack of replications in the experimental set-up.

For identification of the best *Phytophthora* recovery method, analysis was based on the number of *Phytophthora* isolates (*Phytophthora* population*)* from seven leaf baits and number of *Phytophthora* spp. (*Phytophthora* diversity) recovered from seven leaf baits using a one-way ANOVA. For identification of the best bait, the seven leaf baits were evaluated based on the total number of *Phytophthora* isolates and the *Phytophthora* spp. diversity recovered. Initially, a one-way ANOVA was done separately for the laboratory baiting data and the river baiting data for the total number of *Phytophthora* isolates and number of *Phytophthora* spp. obtained from the leaf baits. A second one-way ANOVA of the combined data of the laboratory baiting and river baiting methods were then carried out. If the analysis showed there was a significant difference ($P>0.05$) in the total number of *Phytophthora* isolates or *Phytophthora* spp. recovered from the leaf baits, then this was followed by a pairwise

comparison using Tukey's test of the number of *Phytophthora* isolates or *Phytophthora* spp. from each leaf bait to identify which baits were significantly different. The number of *Phytophthora* isolates obtained on the two *Phytophthora* isolation media (P_5ARPH CMA and P_5ARP.CMA) was analysed using one-way ANOVA.

2.3 Results

2.3.1 Recovery of *Phytophthora* sp. isolates

2.3.1.1 Laboratory and river baiting method

Areas of brown discolouration and water-soaked lesions were observed on the leaf baits obtained from the river and laboratory baiting which indicated that infection had occurred. For *Pi. radiata* and *Ce. deodara* baits, infection typically occurred at the basal or apical tips of the needle (Figure 2.6 A and B) while other leaf baits had lesions localised at the edges of the leaf blade (Figure 2.6 C and D). Infection in *R. arboreum* (Figure 2.7) usually occurred around the midrib vein. No *Phytophthora* strains were isolated from *Ca. japonica* despite lesions being observed and no colonies grew from these lesions on *Phytophthora* selective media.

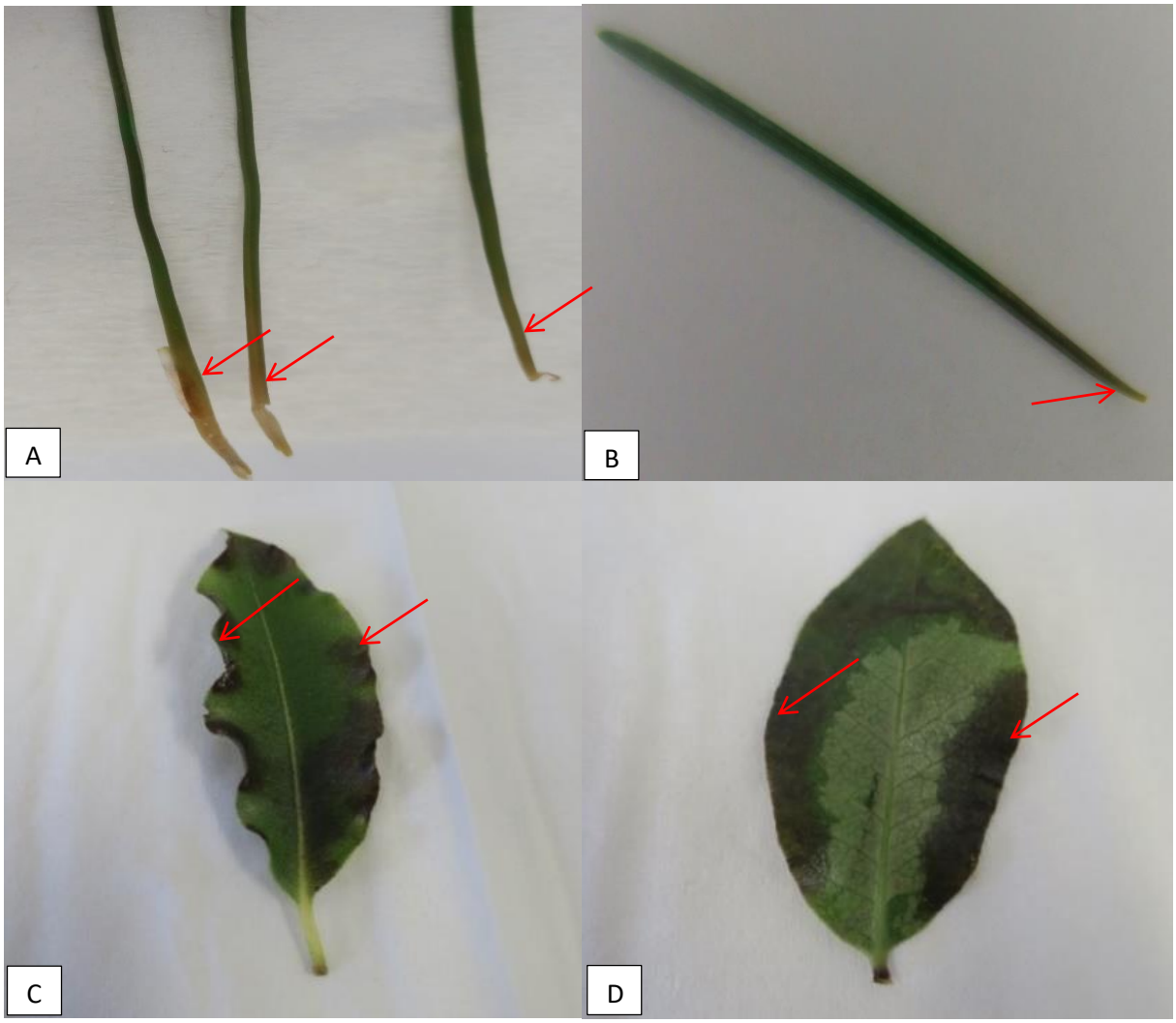

Figure 2.6: Leaf lesions as shown with red arrows on (A) *Ce. deodara,* (B) *Pi. radiata,* (C) *Pt. eugenioides* and (D) *Pt. undulatum.*

Figure 2.7: Leaf lesions surrounding the leaf margin shown with red arrows in *R. arboreum.*

2.3.1.2 Filtration method

Phytophthora colonies appeared after the inverted filter membrane was removed and the isolation media was further incubated. *Phytophthora* spp. colony growth was spread across the plate in areas that were covered by the membrane filter. Only six *Phytophthora* spp. colonies were obtained from the two sites in the Halswell River on isolation media containing hymexazol (PARPH) due to the large number of *Pythium* sp. that grew on the plates. The *Pythium* spp. colonies had a fast growth rate and inhibited the recovery of *Phytophthora* spp. colonies. More *Pythium* growth was observed on PARP (without hymexazol) than PARPH media resulting in no *Phytophthora* colonies recovered on PARP.

2.3.2 Morphological identification of isolates

A total of 391 isolates were obtained from the six sites sampled on the Liffey Stream and Halswell River (Appendix A.2.3) using the three different recovery methods. Two hundred and fifty-six isolates were recovered from the four sites in the Liffey Stream whilst 135 isolates were recovered from the two sites in the Halswell River. The oomycetes *Pythium* and *Phytophthora* were the two most commonly recovered genera from the leaf baits and filter membrane. Sporangia characteristics were used to distinguish between the genera *Phytophthora* and *Pythium.* Isolates with direct release of zoospores from sporangia (Figure 2.8 A) were identified as *Phytophthora*, while *Pythium* (Figure 2.8 B) isolates were characterised by the release of zoospores from sporangia into a vesicle for maturation prior to dispersal.

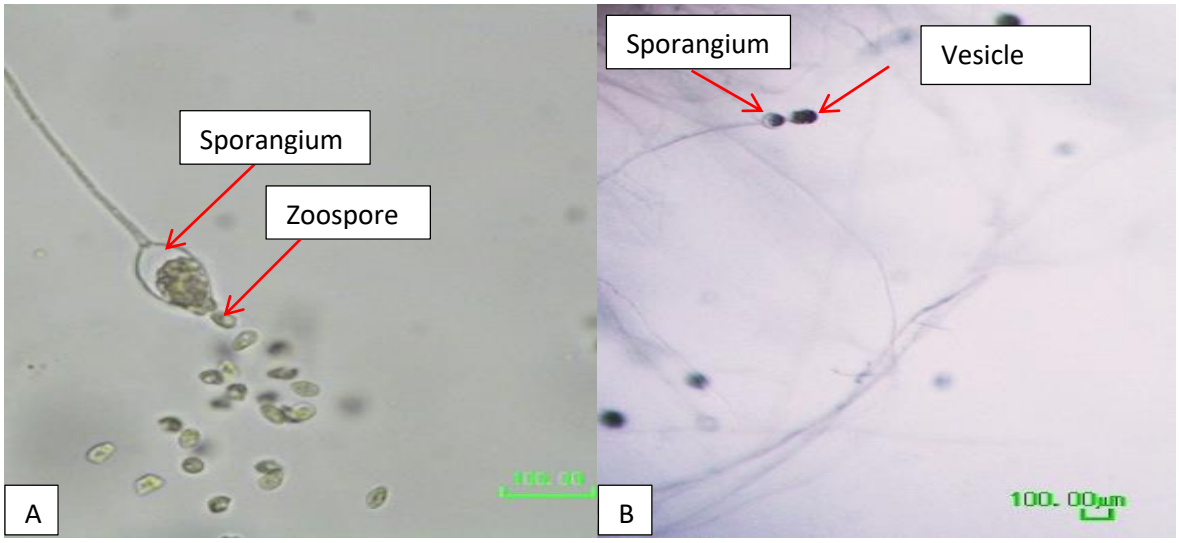

Figure 2.8: Sporangia characteristics; (A) *Phytophthora* sporangium with direct release of zoospores, and (B) *Pythium* sporangium releasing zoospores into a vesicle.

A total of 244 *Phytophthora* spp. isolates were obtained from the six sites (four Liffey Stream, two Halswell River) using a filtration method, laboratory baiting and river baiting. The isolates were assigned into groups initially based on the colony patterns on PDA incubated for 14 days at 20°C in the dark and sporangia characteristics. The molecular identification of the representative isolates from each morphotype gave varied *Phytophthora* spp. indicating that the morphotypes could be divided further to better represent individual *Phytophthora* sp. In addition, measurement of colony growth and patterns were not effective using 14 day old cultures so the colony age was reduced to 7 days. Despite *Phytophthora* spp. showing plasticity in culture, attempts were made to further categorise the isolates into different morphotype groups based on the colony patterns after 7 days growth on PDA and V8A. Colony growth rate was assessed based on the colony diameter as stated in the method (Section 2.2.5).

Sporangial characteristics were examined with shapes including globose (Figure 2.9 A), ovoid (Figure 2.9 A), ellipsoid (Figure 2.9 A), reniform, (Figure 2.9 B), obpyriform (Figure 2.9 C) and obovoid (Figure 2.9 D) observed. Two types of sporangial branching were observed: simple (Figure 2.9 E) and compound (Figure 2.9 F). Sporangia attachment to hypha was either lateral attachment (Figure 2.9 A), basal attachment (Figure 2.9 E) or sub-basal attachment (Figure 2.10 A). Sporangia proliferation was either present or absent. Isolates that had sporangia proliferation had either internal and nested sporangia proliferation (Figure 2.10 B) or internal and extended sporangia proliferation (Figure 2.10 C). Zoospores from sporangia were released from the tip (Figure 2.8 A) of the sporangia. The sporangial tip had structures called papilla (Figure 2.8 B) which were either present or absent in the isolates. In some isolates the sporangia had more than one papilla (Figure 2.10 D).

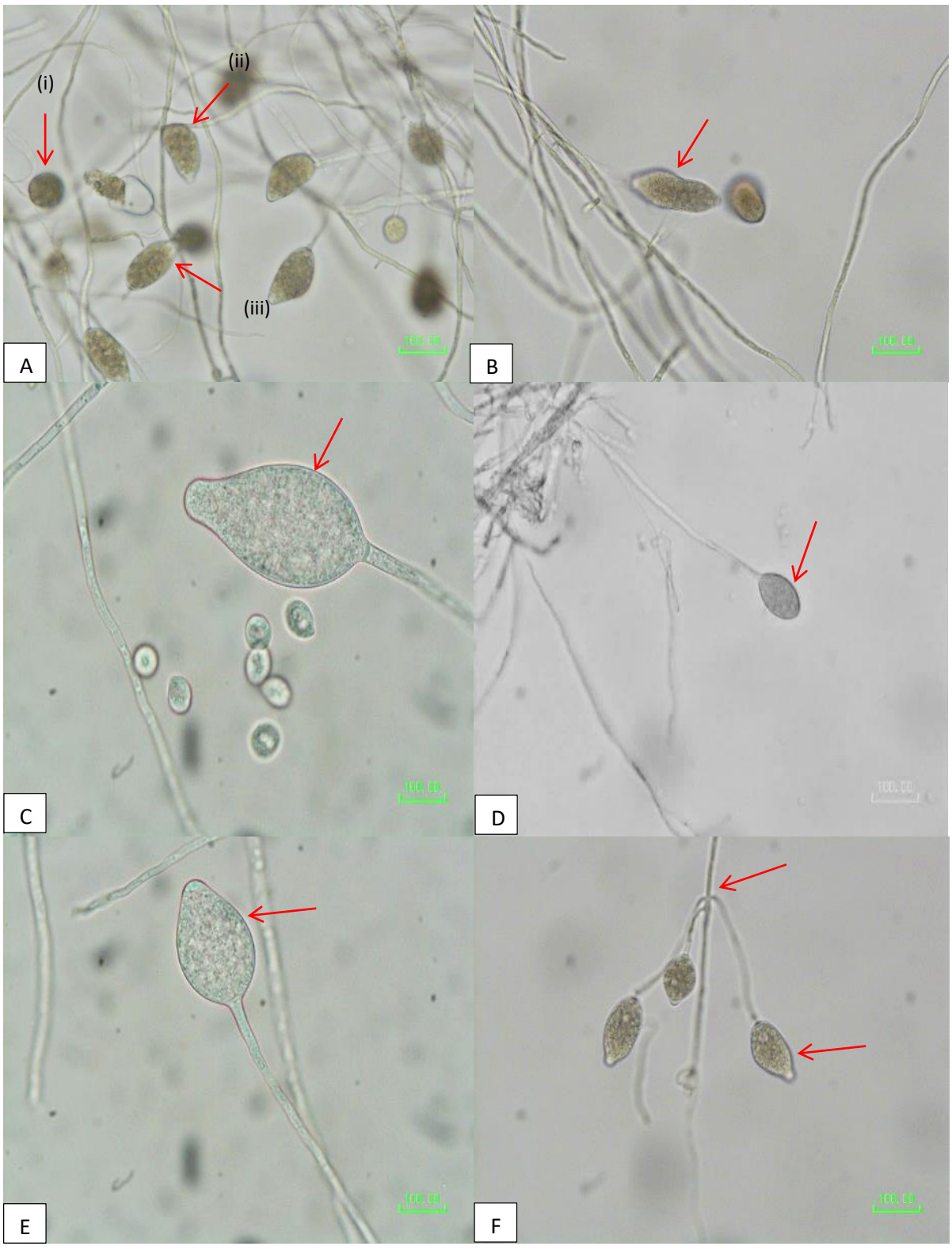

Figure 2.9: Sporangia morphology as shown with red arrows; (A) (i) globose sporangium, (ii) papillate and ovoid sporangium with lateral attachment, (iii) papillate and ellipsoid sporangium (B) reniform sporangium, (C) papillate, obpyriform sporangium, (D) non-papillate obovoid sporangium, (E) non-papillate, ovoid sporangium with simple branching and basal attachment, (F) papillate, ovoid sporangia with basal attachment and compound branching.

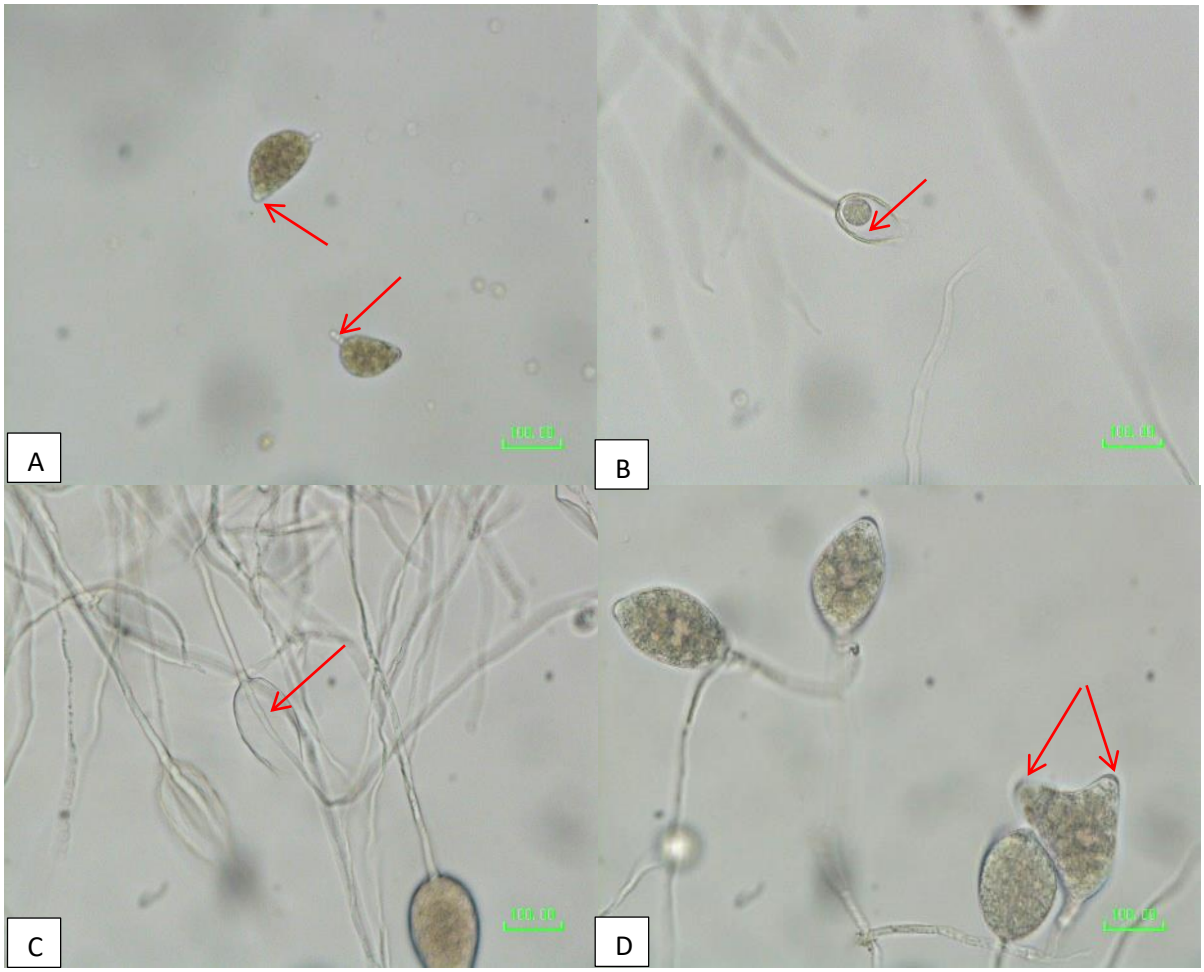

Figure 2.10: Sporangia morphology as shown with red arrows; (A) papillate and ovoid sporangia with sub-basal attachment, (B) internal and nested sporangial proliferation, (C) internal and extended sporangial proliferation, (D) sporangium with more than one papilla.

Based on the colony pattern on PDA and V8A, and sporangia characteristics, the isolates were divided into four morphotype (M1 to M4) groups. Sub-groups were also identified within the morphotype groups due to slight variations in culture morphology.

Morphotype M1

Initially all fast-growing isolates i.e., those that covered the whole plate in 14 days with radiate colony pattern (Figure 2.11) were placed in M1 group. Theses isolates had globose, ellipsoid, ovoid, pyriform and reniform shaped sporangia with compound branching. Sporangia attachment to the hypha was basal and sub-basal attachment with sporangia proliferation absent and more than one papilla present.

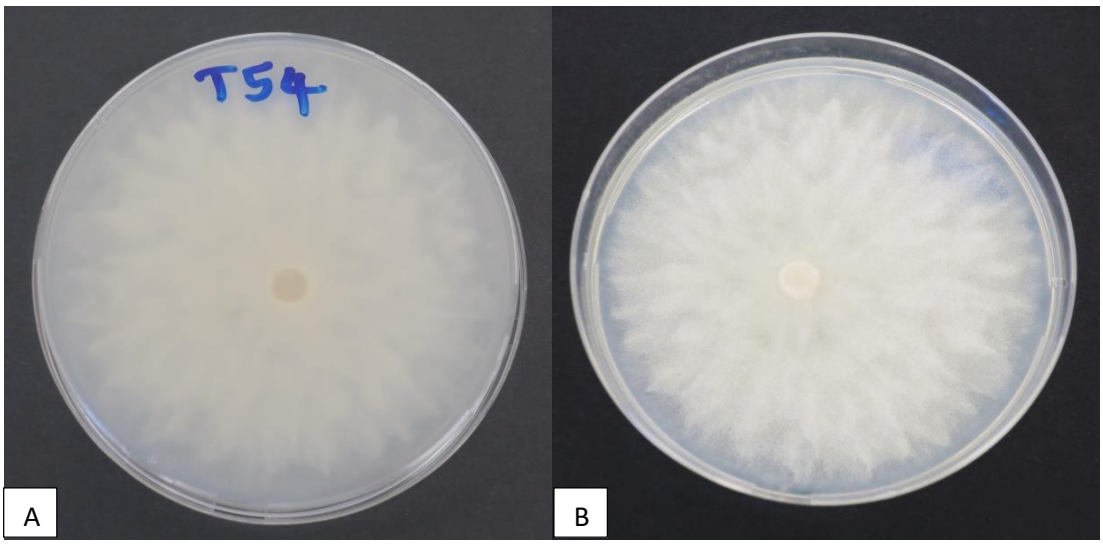

Figure 2.11: Morphotype M1, 14 day-old fast growing radiate colony on PDA; (A) bottom view and (B) top view.

Reducing colony age to 7 days resulted in more isolates from other groups being assigned to the M1 morphotype group and the isolates were separated into three subgroups M1a, M1b and M1c based on growth rates (Section 2.2.6). On PDA the M1a isolates were grouped as having slow growing radiate colonies, with medium growing uniform colonies on V8A (Figure 2.12). While M1b isolates had medium growing radiate colonies on PDA and fast-growing radiate colonies on V8A (Figure 2.13). Isolates grouped into the M1c subgroup had medium growing radiate colonies with medium uniform colonies on V8A (Figure 2.14). All the three subgroups despite having radiating colonies formed slight petal-like shaped colonies on PDA.

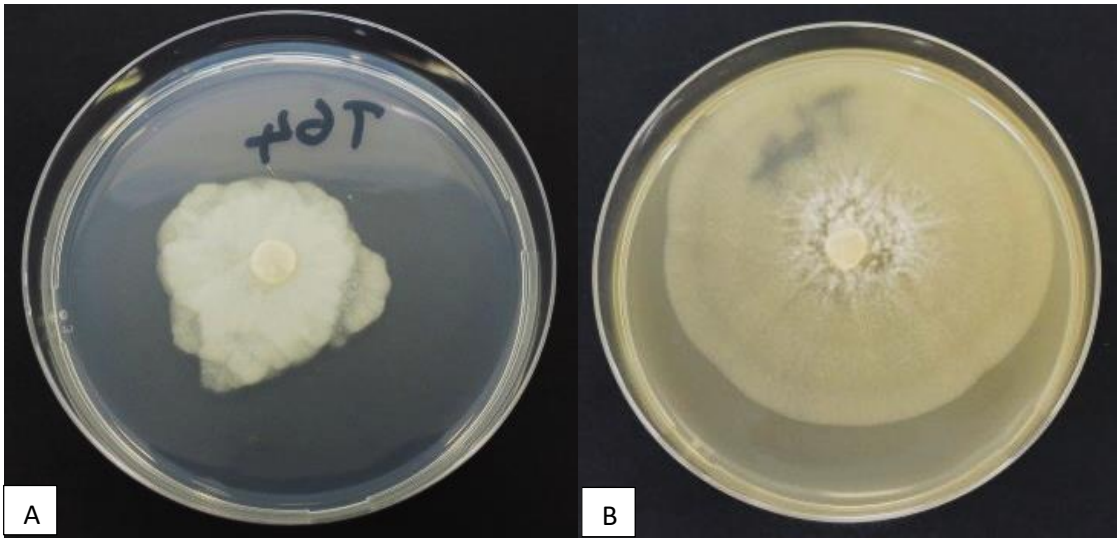

Figure 2.12: Morphotype M1a, 7day-old ; (A) slow growing radiate colony on PDA, and (B) medium growing uniform colony on V8A.

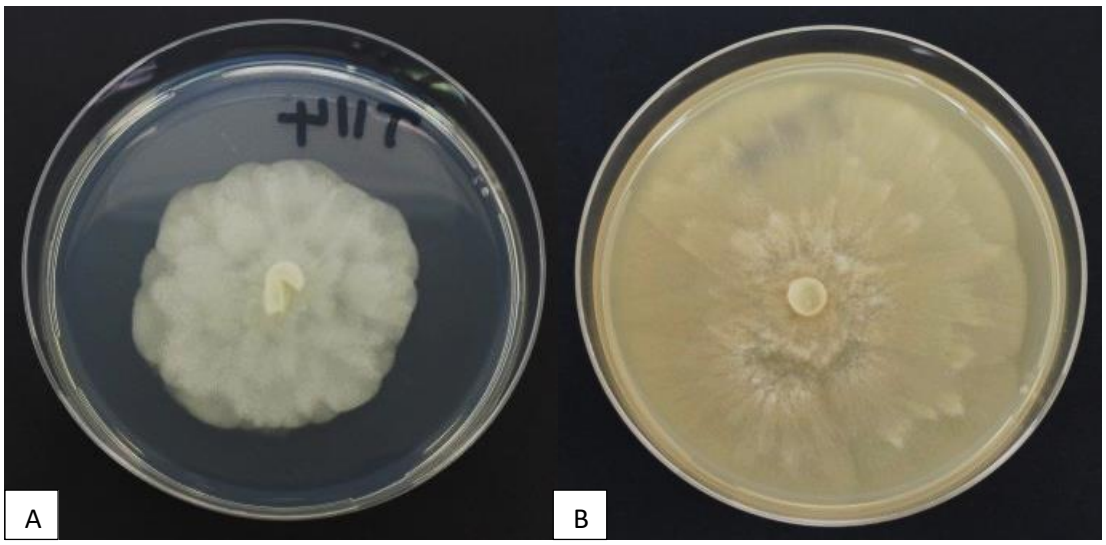

Figure 2.13: Morphotype M1b, 7day-old; (A) medium growing radiate colony on PDA, and (B) fast growing uniform radiate on V8A.

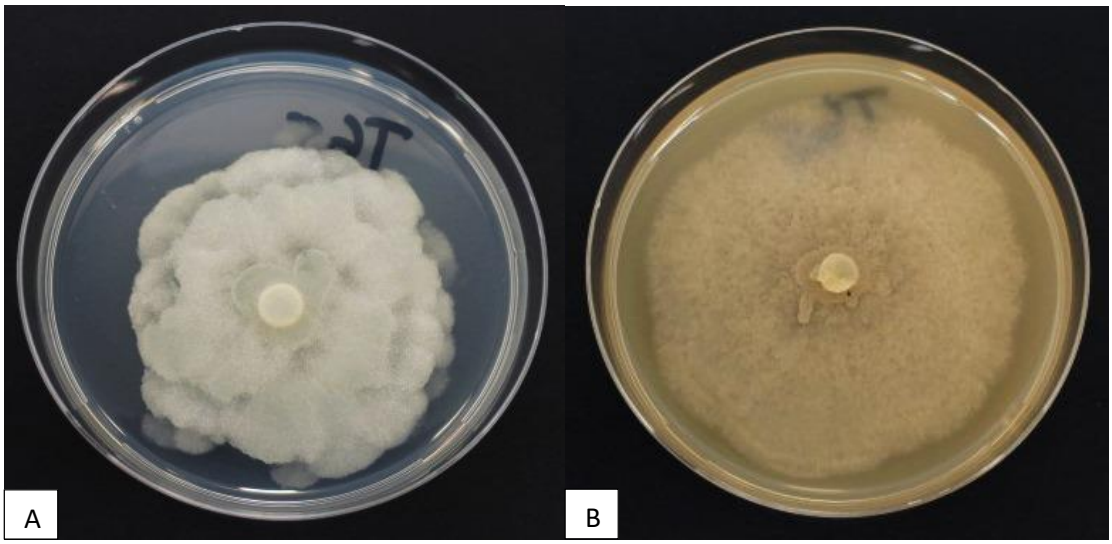

Figure 2.14: Morphotype M1c, 7day-old; (A) medium growing radiating colony on PDA, and (B) medium uniform colony on V8A.

Morphotype M2

Initially isolate grouped within the M2 morphotype were categorised based on having a fast-growing uniform colony pattern on PDA (Figure 2.15) with globose and ovoid sporangial shape. The isolates had compound sporangia branching with basal and sub-basal sporangia attachment to the hypha. Sporangia proliferation was absent and more than one papilla present.

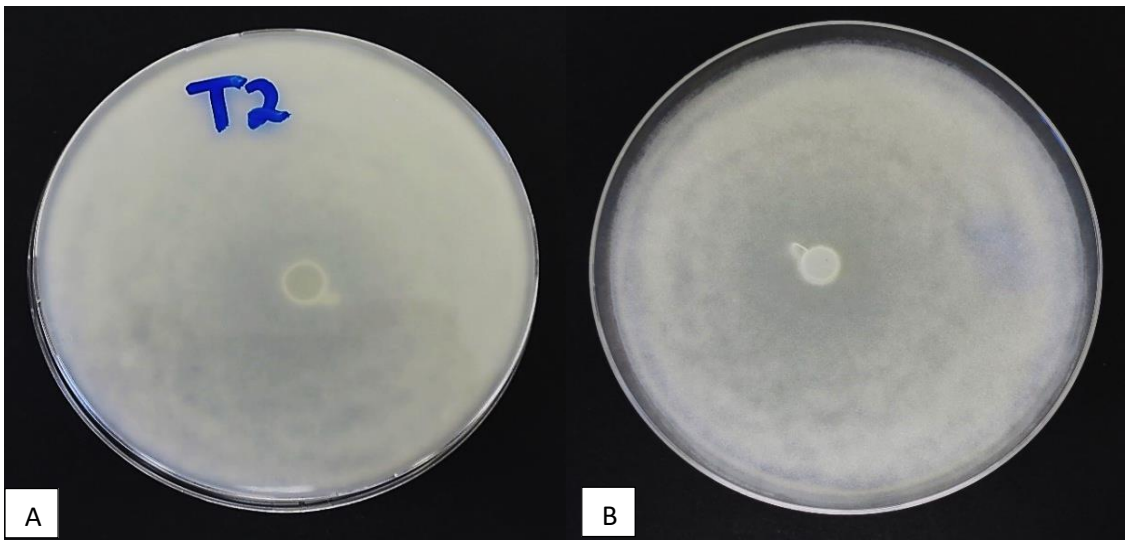

Figure 2.15: Morphotype M2, 14-day old uniform colony on PDA; (A) bottom view and (B) top view.

Based on the morphology of 7-day old colonies on PDA and V8A, this group was divided into two subgroups i.e., M2a and M2b. Isolates grouped as M2a had fast growing uniform colonies on both PDA and V8A (Figure 2.16). Isolates grouped as M2b had fast growing uniform colonies with a slight rosette pattern on PDA and fast-growing uniform colonies on V8A (Figure 2.17). M2a isolates had mostly globose to ovoid sporangia, however M2b isolates had globose, ellipsoid, ovoid and pyriform sporangial shapes. Apart from this, colonies of M2b isolate on PDA had a more transparent and suppressed mycelium growth while M2a isolates had opaque colonies with aerial mycelium growth.

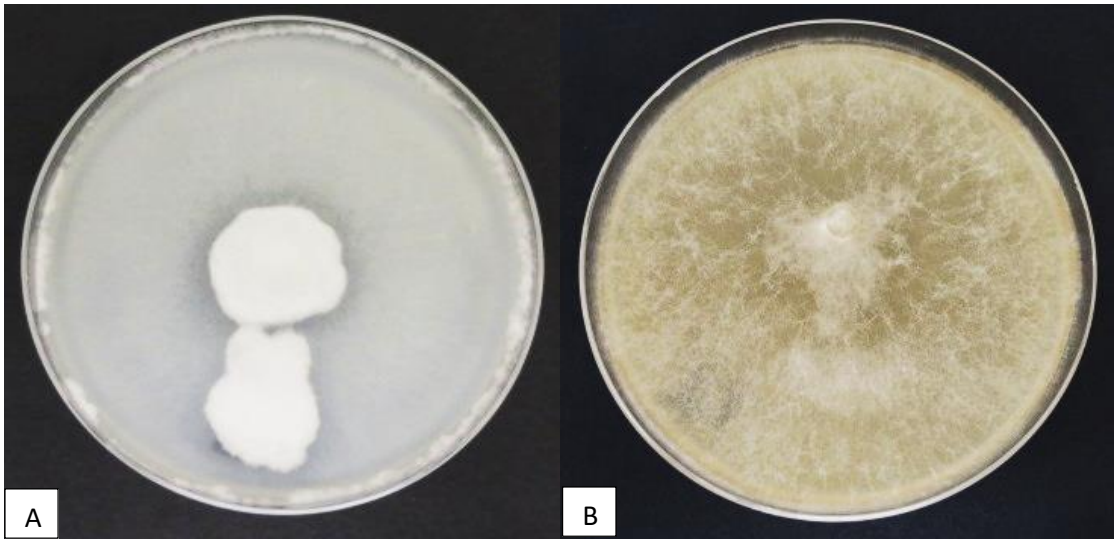

Figure 2.16: Morphotype M2a, 7-day old; (A) fast growing uniform colony on PDA, and (B) fast growing uniform pattern on V8A.

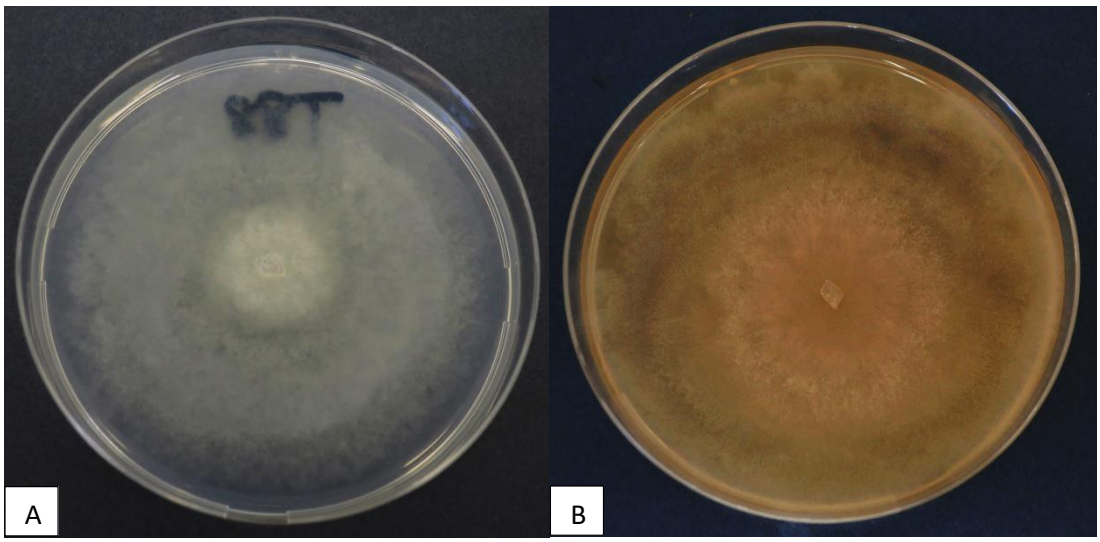

Figure 2.17: Morphotype M2b, 7day-old; (A) fast growing uniform colony with slight rosette pattern on PDA, and (B) fast growing uniform pattern on V8A.

Morphotype M3

Isolates grouped in morphotype M3 had slow growing colonies (Figure 2.18) after 14 days on PDA. These isolates had globose, ellipsoid, ovoid and obpyriform shaped sporangia and simple sporangia branching with basal attachment. Sporangia proliferation was internal, extended and nested with non-papillate sporangia.

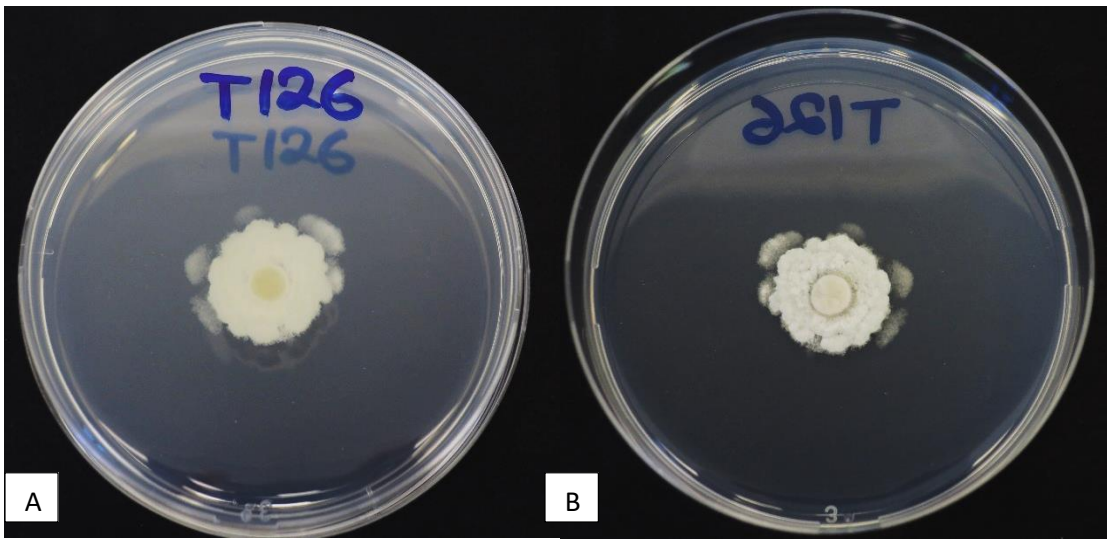

Figure 2.18: Morphotype M3, 14 day-old slow growing uniform colony on PDA; (A) bottom view and (B) top view.

Morphotype M3 was further divided into four subgroups i.e., M3a, M3b, M3c and M3d based on the difference in colony morphology of 7day-old colony on PDA and V8A. Isolates grouped into M3a were slow growing with colonies having fine rosette patterns on PDA, with fast growing colonies with a radiate pattern on V8A (Figure 2.19). Isolates in M3b had very slow growing thick rosette colonies with medium radiating colonies on V8A (Figure 2.20). M3c isolates had very slow growing uniform colonies on PDA with medium uniform colonies on V8A (Figure 2.21). Isolates grouped into M3d had slow growing rosette colonies on PDA and medium uniform colonies on V8A (Figure 2.22).

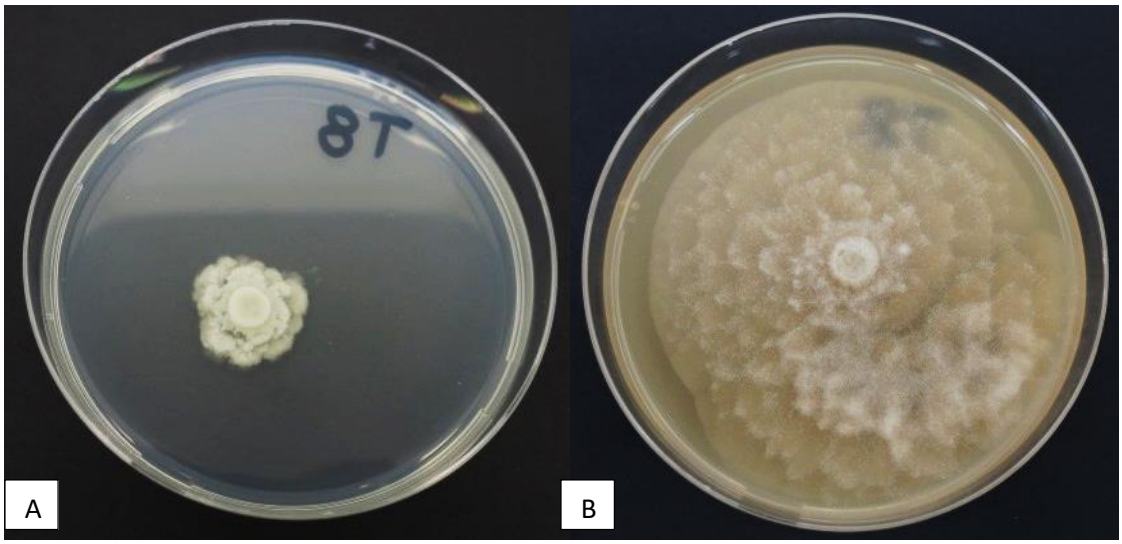

Figure 2.19: Morphotype M3a, 7day-old; (A) slow growing rosette colony on PDA, and (B) fast growing radiate pattern on V8A.

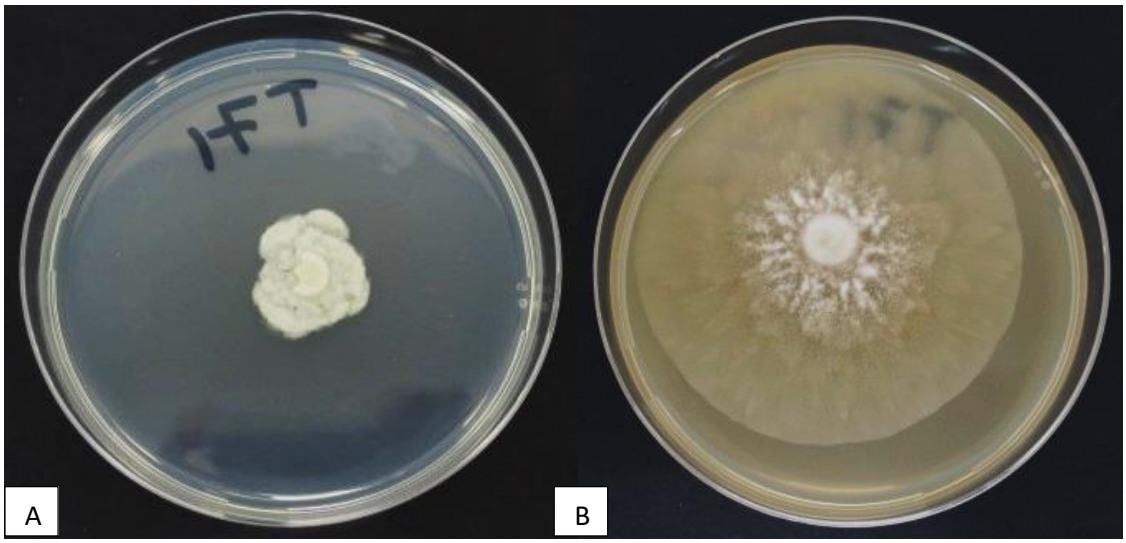

Figure 2.20: Morphotype M3b, 7 day-old; (A) very slow growing rosette colony on PDA, and (B) medium radiating colony on V8A.

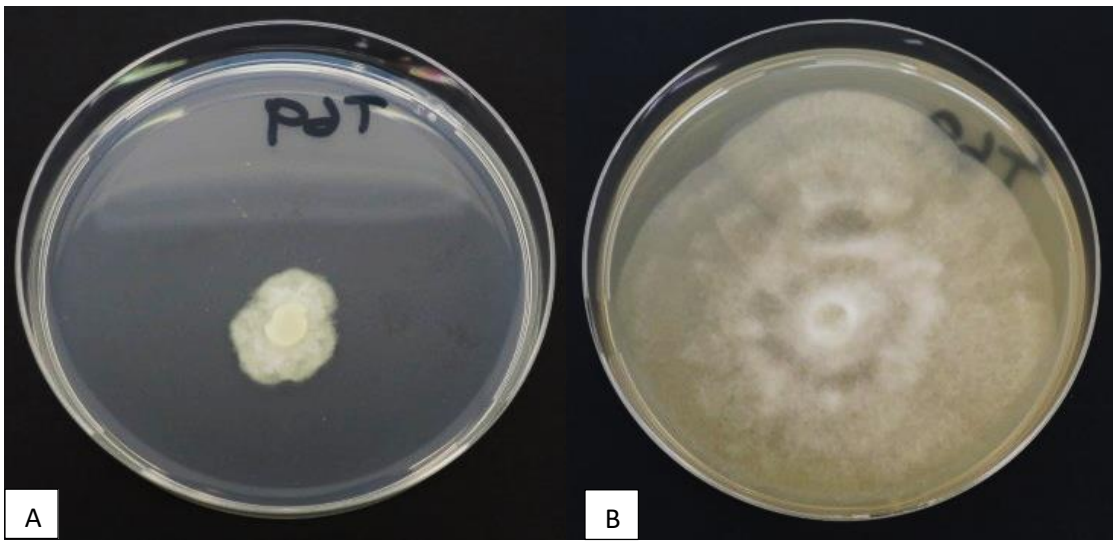

Figure 2.21: Morphotype M3c, 7 day-old; (A) very slow growing uniform colony on PDA, and (B) medium uniform colony on V8A.

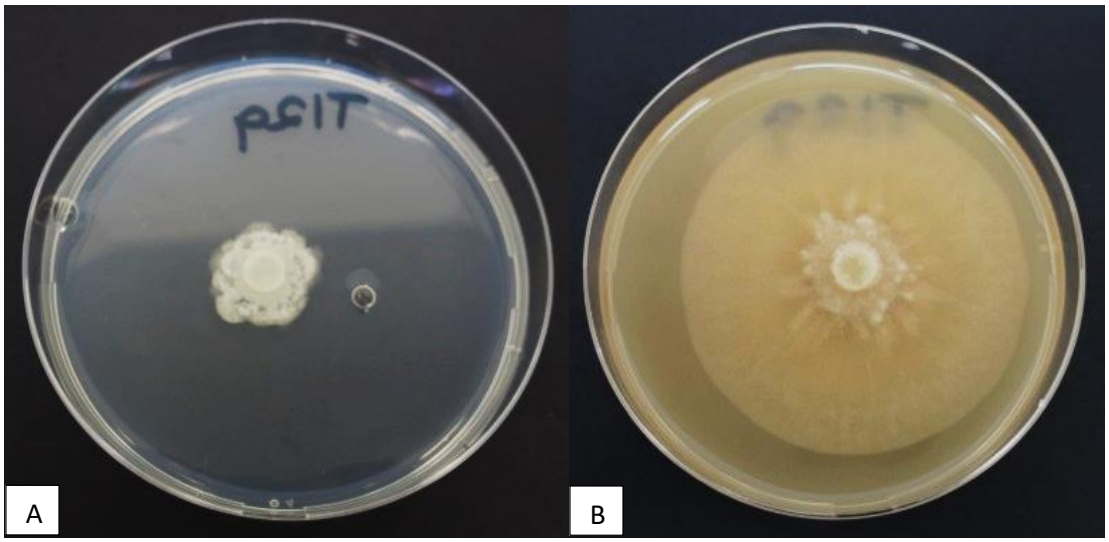

Figure 2.22: Morphotype M3d, 7 day-old; (A) slow growing rosette colony on PDA, and (B) medium uniform colony on V8A.

Morphotype M4

Isolates in morphotype M4 had fast growing rosette colonies (Figure 2.23) with globose, ellipsoid, ovoid and pyriform shaped sporangia. Sporangia had simple branching with basal attachment, internal, extended and nested proliferation and non-papillate sporangia.

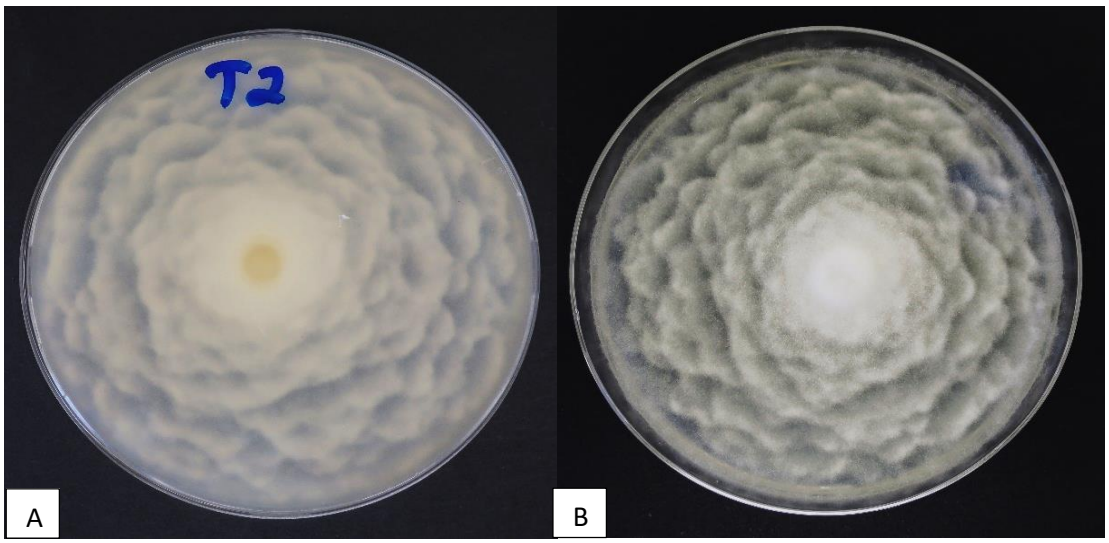

Figure 2.23: Morphotype M4, 14 day-old fast growing rosette colony on PDA; (A) bottom view and (B) top view.

Based on the morphology of 7 day-old PDA and V8A colonies, morphotype M4 was split into three sub-groups i.e., M4a, M4b and M4c. Isolates grouped into M4a had medium growing rosette colonies on PDA with medium radiate colonies on V8A (Figure 2.24). M4b isolates had medium growing rosette colonies on PDA and medium uniform colonies on V8A (Figure 2.25). Isolates grouped into M4c had fast growing rosette colonies on PDA and fast growing rosette colonies on V8A (Figure 2.26).

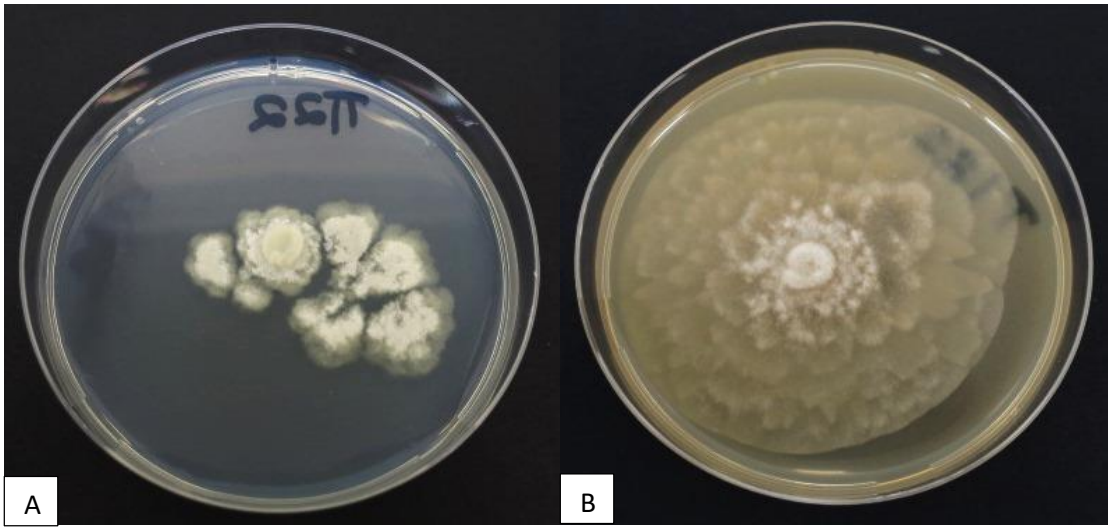

Figure 2.24: Morphotype M4a, 7 day-old; (A) slow growing rosette colony on PDA, and (B) medium uniform colony on V8A.

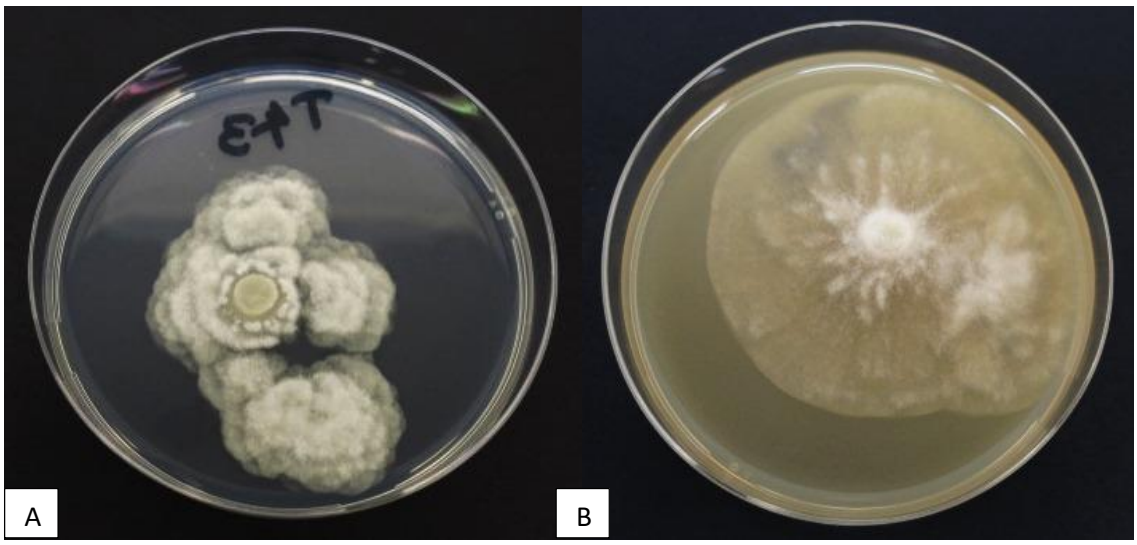

Figure 2.25: Morphotype M4b, 7 day-old; (A) medium growing rosette colony on PDA, and (B) medium uniform colony on V8A.

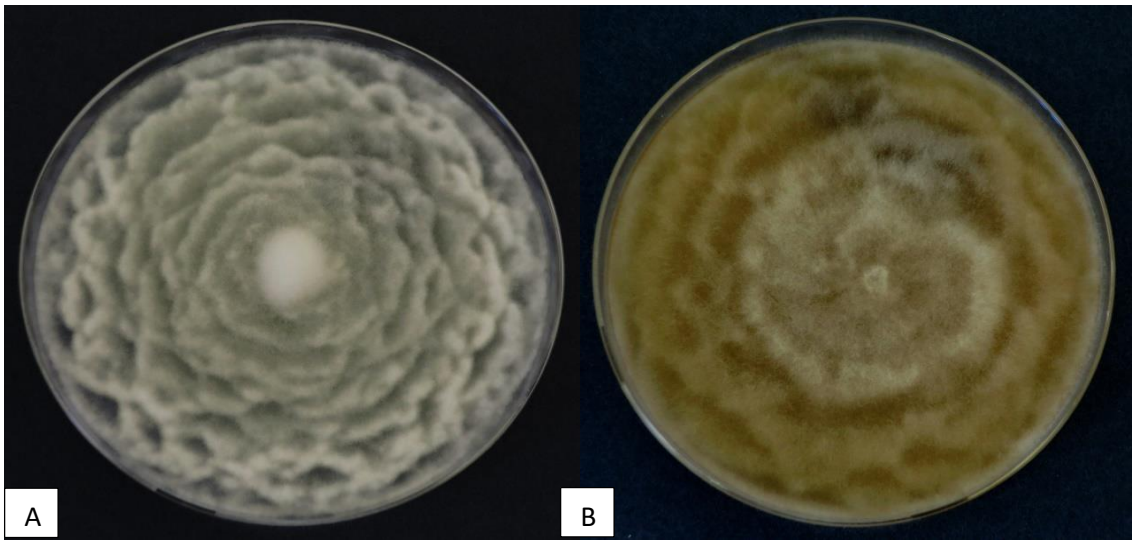

Figure 2.26: Morphotype M4c, 7 day-old; (A) fast growing rosette colony on PDA, and (B) fast growing fine rosette colony on V8A.

Despite the variations in culture morphologies within the morphotypes that gave rise to subgroups, DNA identification (Section 2.3.3) showed that overall the subgroups were the same *Phytophthora* spp. The variation was due to plasticity of *Phytophthora* spp. in culture.

2.3.3 *Phytopththora* sp. DNA identification

For each morphotype approximately 10% to 20% of isolates were randomly selected for DNA identification (Appendix A.2.5). The ITS results were used to confirm that the isolates from the morphotype groups were *Phytophthora* spp. and isolates from each ITS group was selected for

confirmation of the *Phytophthora* spp. using the sequence of the *coxI* gene (Table 2.2). From the two sites in the Halswell River, six *Phytophthora* spp. were confirmed using *coxI*. These included *Phytophthora* sp. LS-2018c strain CL 181, *Ph. cactorum, Ph. gonapodyides, Ph. lacustris*, *Ph. cryptogea,* and a *Ph. thermophila x amnicola* hybrid. Three *Phytophthora* spp. was recovered from the Liffey Stream i.e., *Ph. lacustris, Ph. thermophila x amnicola* hybrids and *Ph. amnicola x Phytophthora* taxon Pgchlamydo hybrids. *Phytophthora* taxon Pgchlamydo hybrid has been redesignated *Phytophthora chlamydospora* (Hansen *et al.*, 2015).

The results of the identification of the *Phytophthora* isolates from the different morphological groups is shown in Table 2.2. A total of 30 isolates in morphotype M1, across all three subgroups were identified as *Phytophthora* sp. LS-2018c strain CL 181 using *coxI*. These isolates were only recovered from the two sites in the Halswell River. Morphotype M2 had only three isolates and the one isolate in subgroup M2b was identified as *Ph. cactorum* which was obtained from site 2 in the Halswell River. Subgroup M2a was identified as *Pythium* and therefore excluded from further analysis. Morphotype M3 was the largest group with 160 isolates obtained from all the six sites sampled. The majority of the M3 isolates were identified as *Phytophthora* hybrids i.e., *Ph. thermophila x amnicola* hybrid and *Ph. amnicola x Ph. chlamydospora* hybrid. However, isolates identified as *Ph. gonapodyides* were also found in this group which indicated that morphotype M3 isolates were a mixture of *Phytophthora* hybrids and *Ph. gonapodyides.* Morphotype M4 had a total of 33 isolates that was divided into three subgroups. Morphotype M4a had only two isolates identified as *Ph. lacustris* and *Ph. cryptogea* obtained from Halswell river site 1 and site 2, respectively. While the remaining isolates of the M4 subgroups were identified as *Ph. lacustris.*

Table 2.2: Identification of the *Phytophthora* isolates from the different morphological groups obtained from two sites in the Halswell River and four sites in the Liffey Stream based on sequencing of the ITS and *coxI* gene regions. For each isolate identification code, 'T' represents isolates from Halswell River and 'LIF' represents isolates from Liffey Stream.

Morphotype	**Sub-groups**	**Isolate ID**	**DNA identification**			
			ITS ID	**Accession**	***coxI* ID**	**Accession**
M1 (30 isolates)	M1a (18 isolates)	T6	*Ph. siskiyouensis* (99%)	EF490682.1	*Phytophthora* sp. LS-2018c strain CL 181(99%)	MG721489.1
		T5	*Ph. siskiyouensis* (99%)	EF490682.1	*Phytophthora* sp. LS-2018c strain CL 181(100%)	MG721489.1
		T52	*Ph. siskiyouensis* (98%)	EF490682.1	*Phytophthora* sp. LS-2018c strain CL 181(99%)	MG721489.1
		T73	*Ph. siskiyouensis* (99%)	EF490682.1	-	
		T3	-		*Phytophthora* sp. LS-2018c strain CL181 147-999bp	MG721489.1
	M1b (9 isolates)	T33	*Ph. siskiyouensis* (99%)	EF490682.1	*Phytophthora* sp. LS-2018c strain CL181 (99%)	MG721489.1
		T28	*Ph. siskiyouensis* (99%)	EF490682.1	*Phytophthora* sp. LS-2018c strain CL181 (99%)	MG721489.1
	M1c (3 isolates)	T65	*Ph. plurivora* (99%)	KC602469.1	*Phytophthora* sp. LS-2018c strain CL181 (99%)	MG721489.1
		T113	*Ph. siskiyouensis* (99%)	EF490682.1	*Phytophthora* sp. LS-2018c strain CL181 (99%)	MG721489.1
M2 (3 isolates)	M2a (2 isolates)	T1, T75			*Pythium dissotocum* (99%)	KJ595479.1
	M2b (1 isolate)	T88	-		*Ph. cactorum* (99%)	AY129174.1

Table 2.2 continued

Morphotype	Sub-groups	Isolate ID	DNA identification			
			ITS ID	Accession	*coxI* ID	Accession
M3 (160 isolates)	M3a (75 isolates)	T95	*Ph. gonapodyides* (98%)	HM004231.1	*Ph. thermophila x Ph. amnicola* strain MUCC780 (100%)	JQ936803.1
		T24	*Ph. gonapodyides* (98%)	HM004231.1	*Ph. gonapodyides* (100%)	MG721475.1
		T128	*Ph. gonapodyides* (99%)	HM004231.1		
		T125	*Ph. gonapodyides* (98%)	HM004231.1		
	M3b (37 isolates)	T29	*Ph. gonapodyides* (98%)	HM004231.1	*Ph. thermophila x Ph. amnicola* strain MUCC780 (100%)	JQ936803.1
		T35	-		*Ph. thermophila x Ph. amnicola* strain MUCC780 (100%)	JQ936803.1
		LIF 18	*Phytophthora* taxon PgChlamydo (96%)*	HM004224.1	*Ph. thermophila x Ph. amnicola* strain MUCC781 (100%)	JQ936804.1
		LIF 213	*Phytophthora* taxon Pgchlamydo (98%)*	KJ755194.1	*Ph. thermophila x Ph. amnicola* strain MUCC781 (100%)	JQ936804.1
		T32			*Ph. thermophila x Ph. amnicola* strain MUCC781 (100%)	JQ936804.1
		LIF 180	*Ph. gonapodyides* (98%)	HM004231.1	*Ph. thermophila x Ph. amnicola* strain MUCC780 (100%)	JQ936803.1
		LIF 98	*Ph. gonapodyides* (99%)	HM004231.1		
		LIF 89	*Ph. gonapodyides* (99%)	HM004231.1	*Ph. amnicola x Phytophthora* taxon *Pgchlamydo* strain* CMW37730 (100%)	JQ890351.1

Table 2.2 continued

Morphotype	Sub-groups	Isolate ID	DNA identification			
			ITS ID	Accession	*coxI* ID	Accession
M3	M3b	LIF 109	*Ph. gonapodyides* (99%)	HM004231.1	*Ph. thermophila x Ph. amnicola* strain MUCC780 (99%)	JQ936803.1
		LIF 192	*Ph. gonapodyides* (99%)	HM004231.1		
		LIF 231	*Ph. gonapodyides* (99%)	HM004231.1		
		LIF 224	*Ph. gonapodyides* (99%)	HM004231.1		
		LIF 39	*Ph. gonapodyides* (99%)	HM004231.1		
		LIF 16	*Ph. gonapodyides* (99%)	HM004231.1		
		LIF 21	*Ph. gonapodyides* (99%)	HM004231.1		
		LIF 64	*Ph. gonapodyides* (99%)	HM004231.1		
		LIF 50	*Ph. gonapodyides* (99%)	HM004231.1		
	M3c (27 isolates)	T45	*Ph. humicola* (96%)	JQ757060.1	*Ph. thermophila x Ph. amnicola* strain MUCC781 (100%)	JQ936804.1
	M3d (21 isolates)	T8	*Ph. borealis* (98%)	JQ626599.1	*Ph. thermophila x Ph. amnicola* strain MUCC780 (100%)	JQ936803.1
		T58	*Ph. borealis* (98%)	JQ626599.1	*Ph. thermophila x Ph. amnicola* strain MUCC781 (99%)	JQ936804.1
		T101	*Ph. borealis* (98%)	JQ626599.1		
M4 (33 isolates)	M4a (2 isolates)	T46	-		*Ph. lacustris* (100%)	JQ626633.1
		T100	-		*Ph. cryptogea* isolate P1380 (100%)	KJ469046.1
	M4b (20 isolates)	T43	-		*Ph. lacustris* (100%)	HQ012880.1
		T44	*Ph. lacustris* (100%)	HM004219.1	-	

Table 2.2 continued

Morphotype	Sub-groups	Isolate ID	DNA identification			
			ITS ID	Accession	*coxI* ID	Accession
	M4c (11 isolates)	LIF 42	*Ph. lacustris* (99%)	HM004219.1	*Ph. lacustris* (100%)	JQ626633.1
		LIF 19	*Ph. lacustris* (99%)	HM004219.1		
		LIF 13	*Ph. lacustris* (100%)	HM004219.1	*Ph. lacustris* (100%)	JQ626633.1

**Phytophthora* taxon Pgchlamydo has been redesignated as *Phytophthora chlamydospora* (Hansen *et al.*, 2015).

From the Liffey Stream a total of 150 *Phytophthora* isolates were recovered from the four sites, of which 82.4% were identified as *Ph. thermophila x Ph. amnicola* and *Ph. amnicola x Ph. chlamydospora* hybrids. The remaining 17.6% of isolates were identified as *Ph. lacustris*. The same *Phytophthora* species were recovered from all four sites in the Liffey Stream. Site 1 was in the stream in the Lincoln township running through a patch of native trees while site 2 (0.35 km away from site 1) was also in the Lincoln township area and had similar vegetation. Site 3 (0.39 km away from site 2) and site 4 (0.31 km from site 3) were both located in the residential part of Lincoln with vegetation similar to sites 1 and 2.

From the Halswell River, *Phytophthora* sp. LS-2018c strain CL181 (23 isolates), *Ph. thermophila x Ph. amnicola* hybrids (29 isolates) and *Ph. lacustris* (six isolates) were the most commonly recovered spp. A low number of isolates (one isolate each) of *Ph. gonapodyides*, *Ph. cactorum* and *Ph. cryptogea* were recovered. The two sites sampled in the Halswell River were approximately 2.24 km distance apart with differing land use. Site 1 passed through the Tai Tapu village and therefore had greater vegetation variability due to varying plants grown in gardens by the residents. Site 2 was mostly surrounded by sheep, dairy and pasture with some residential properties beside the sampling area. *Phytophthora* sp. LS-2018c strain CL181, *Ph. thermophila x Ph. amnicola* hybrids, *Ph. gonapodyides* and *Ph. lacustris* were recovered from both sites 1 and 2. While, *Ph. cactorum* and *Ph. cryptogea* were only found in site 2.

2.3.4 Identification of the best method and best leaf bait and for baiting *Phytophthora* spp.

Analysis for identification of the best baiting method and leaf baits was performed using only results from the two sites in Halswell River by analysing the total number of *Phytophthora* isolates and *Phytophthora* spp. obtained. The Liffey Stream baits were not included in the analysis as they had been disturbed by the public, which resulted in decay of some the leaf baits. *Camellia japonica* bait did not recover any *Phytophthora* isolates and was not included in the analysis.

2.3.4.1 Identification of the best method for recovery of *Phytophthora* spp.

The three methods used for isolating *Phytophthora* spp. in the Liffey Stream (four sites) gave a total of 17 *Phytophthora* isolates from the filtration method, 76 isolates from the laboratory baiting and only 57 isolates from the river baiting as most of the baits had decayed. The Filtration method recovered *Ph. lacustris* and *Ph. thermophila x amnicola* hybrids, while the laboratory and river baiting recovered three species. i.e., *Ph. lacustris, Ph. thermophila x amnicola* hybrids and *Ph. amnicola x Ph. chlamydospora* hybrids.

In the Halswell River, river baiting recovered a total of 31 isolates from the two sites and laboratory baiting isolated 27 isolates. River baiting recovered five *Phytophthora* spp. i.e., *Ph. thermophila x P. amnicola* hybrids (17 isolates), *Phytophthora* sp. LS-2018c strain CL 181 (nine isolates), *Ph. lacustris* (three isolates), *Ph. cactorum* (one isolate) and *Ph. cryptogea* (one isolate). The laboratory baiting recovered four *Phytophthora* spp. i.e., *Ph. thermophila x P. amnicola* hybrids (21 isolates), *Ph. gonapodyides* (one isolate), *Phytophthora* sp. LS-2018c strain CL 181 (three isolates) and *Ph. lacustris* (two isolates).

For the Halswell River, the filtration method isolated five *Phytophthora* isolates and two *Phytophthora* spp. i.e., *Phytophthora* sp. LS-2018c strain CL181 (four isolates) and one *Ph. lacustris* isolate on media containing hymexazol (P_5ARPH CMA). No *Phytophthora* isolates could be recovered on media without hymexazol (P_5ARP CMA). The low number of *Phytophthora* isolated from the filtration method was due to the large number of *Pythium* spp. and other faster growing oomycetes which overgrew the *Phytophthora* colonies. Isolation from such colonies resulted in contaminated cultures which could not be used for *Phytophthora* identification.

The laboratory and river baiting methods were analysed to identify if river baiting recovered a greater number of *Phytophthora* isolates when compared to laboratory baiting. There was no significant difference (P=0.189; Appendix A.2.6; Table A1) in the number of *Phytophthora* isolates obtained from the two methods. Similarly, there was no significant difference (P=0.169; Appendix A.2.6; Table A2) in the total number of *Phytophthora* spp. isolated from the two baiting methods.

2.3.4.2 Identification of the best leaf bait for baiting for *Phytophthora* spp.

Across the two baiting methods, the greatest number of *Phytophthora* isolates were recovered from *R. arboreum* (49%); followed by *Pi. radiata* (18%), *Pt. undulatum* (14%), *Ce. deodara* (8%), *B. attenuata* (8%) and *Pt. eugenioides* (3%). While no *Phytophthora* isolate was obtained from *Ca. japonica* leaf baits (Figure 2.27).

When the number of *Phytophthora* isolates obtained for the leaf bait types in the laboratory baiting method was analysed, the bait type seemed to affect the number of *Phytophthora* isolates recovered although the effect was marginally not statistically significant (P=0.060) (Figure 2.27; Appendix A.2.6, Table A3). In the river baiting method, there was a significant effect (P =0.026; Appendix

A.2.6; Table A4) of bait type on the total number of *Phytophthora* isolates recovered from the seven leaf bait types. Analysis of the combined data for the laboratory and river baiting also showed that there was a significant effect (P=0.006; Appendix A.2.6; Table A5) of leaf bait type on the number of *Phytophthora* isolates recovered. Tukey's test revealed that the number of *Phytophthora* isolates recovered from *R. arboreum* was significantly higher from the other leaf baits (P=0.021; Appendix A.2.6; Table A6).

The seven leaf baits were also evaluated based on the total number of *Phytophthora* spp. isolated over the two baiting methods i.e., laboratory and river baiting. *Pittosporum undulatum* recovered the highest number of *Phytophthora* spp. (four spp.; *Ph. cactorum, Ph. lacustris, Ph. gonapodyides* and *Ph. thermophila x amnicola* hybrids) followed by *Rhododendron arboreum* which recovered three spp. (*Phytophthora* sp. LS 2018c strain CL 181, *Ph. thermophila x amnicola* hybrids and *Ph. cryptogea), Ce. deodara* recovered three *Phytophthora* spp. (*Phytophthora* sp. LS 2018c strain CL 181, *Ph. lacustris* and *Ph. thermophila x amnicola* hybrids) and *Pi. radiata* which recovered two *Phytophthora* spp. (*Phytophthora* sp. LS 2018c strain CL 181 and *Ph. thermophila x amnicola* hybrids). *Pittosporum eugenioides* recovered two *Phytophthora* spp. (*Phytophthora* sp. LS 2018c strain CL 181and *Ph. thermophila x amnicola* hybrids), while *B. attenuata* recovered only one *Phytophthora* spp. (*Ph. thermophila x amnicola* hybrids). No *Phytophthora* isolate was recovered from *Ca. japonica* (Figure 2.28).

Statistical analysis showed that there was no significant difference in the number of *Phytophthora* spp. isolated from the leaf bait types in river baiting method (P=0.167; Appendix A.2.6; Table A8) or the laboratory baiting method (P=0.099; Appendix A.2.6; Table A7). Analysis of the combined data from the two methods also showed that there was also no significant difference in the number of *Phytophthora* spp. isolated from the leaf bait types (P=0.124; Appendix A.2.6, Table A9).

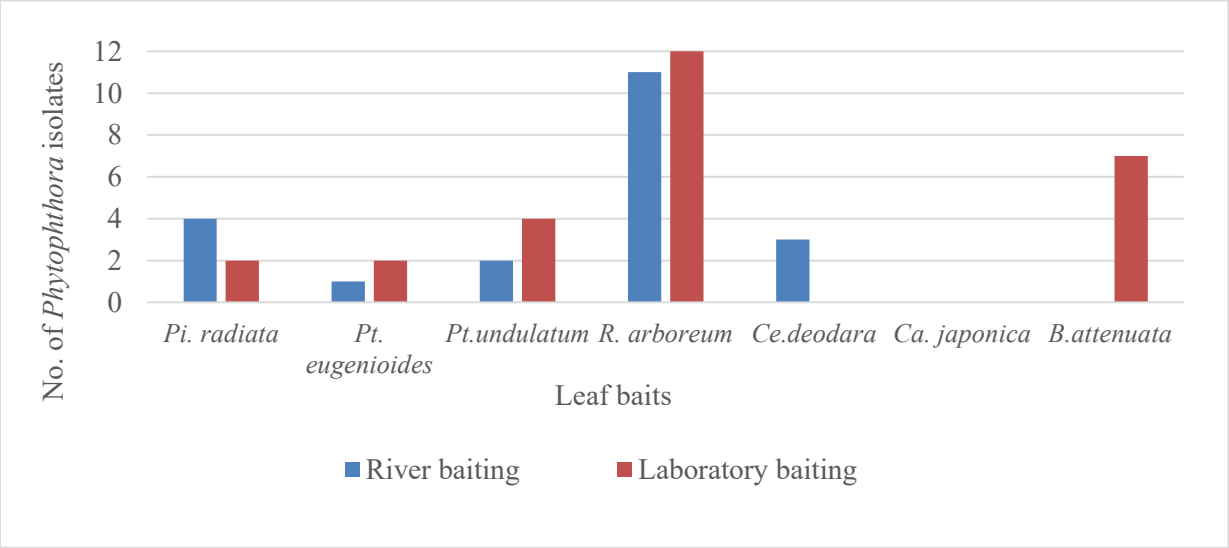

Figure 2.27: Total number of *Phytophthora* isolates obtained from the different leaf baits for the two baiting methods.

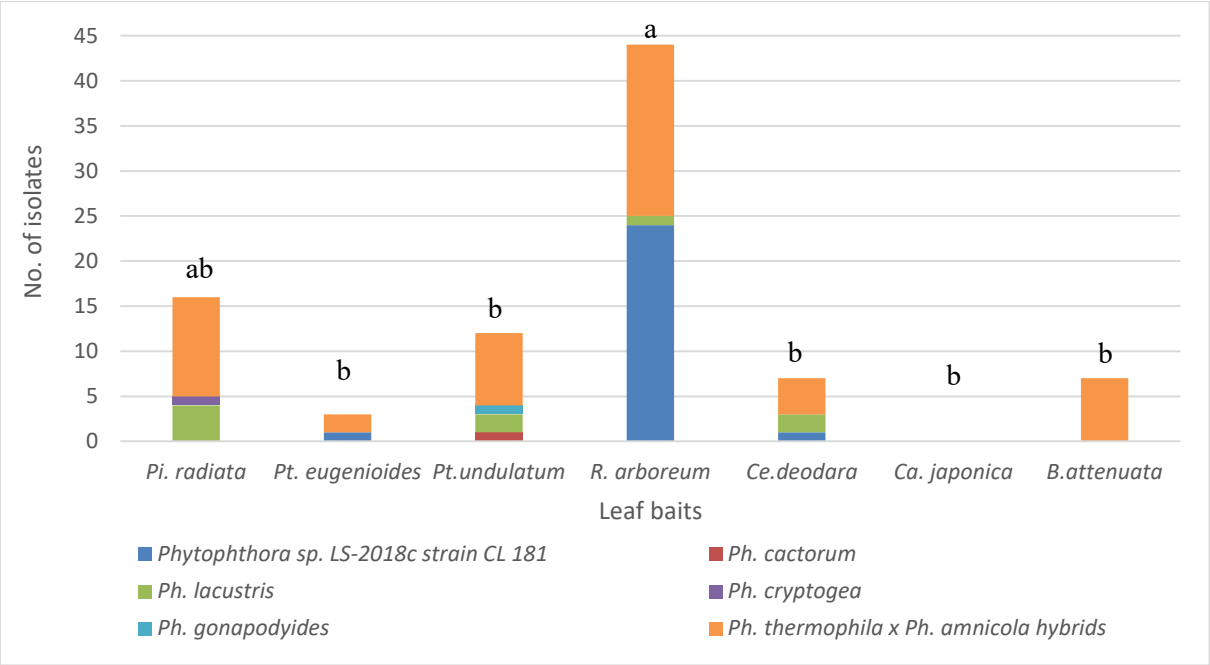

Figure 2.28: Number of isolates of the different *Phytophthora* spp. isolated from the different leaf baits. The different letters above the column indicate statistical significance (P=0.05 using Tukey's honestly significant difference (HSD).

2.3.5 Identification of the best *Phytophthora* isolation media

Phytophthora isolation media PARPH and PARP was also evaluated to determine if the additon of hymexazol, which inhibits some of the *Pythium* spp., could be used to recover a greater number of *Phytophthora* isolates. For the two media, PARP with no hymexazol isolated 58% while PARPH isolated 42% of the *Phytophthora* isolates. Statistical analysis showed that there was no significance difference (P= 0.50, Appendix A.2.5, Table A10) in the number of *Phytophthora* isolates obtained from PARP and PARPH isolation media. Both PARP and PARPH isolated the three most commonly isolated *Phytophthora* spp. i.e., *Phytophthora* sp. LS-2018c strain CL181, *Ph. thermophila x Ph. amnicola* hybrids and *Ph. lacustris.* While PARP also isolated *Ph. cactorum, Ph. gonapodyides* and *Ph. cryptogea.*

2.4 Discussion

2.4.1 *Phytophthora* baiting in waterways

This research aimed to identify the diversity of *Phytophthora* spp. in Canterbury waterways and is the first such study to be conducted for the South Island in New Zealand. This Chapter identified the best method and leaf baits that could be used for more extensive *Phytophthora* baiting in a river system. Seven *Phytophthora* spp. were recovered from the six sites surveyed in two waterways (Liffey Stream and Halswell River) in the summer using three baiting methods (river baiting, laboratory baiting and filtration), being *Ph. thermophila x Ph. amnicola* hybrid (153 isolates), *Ph. amnicola x Ph. chlamydospora* hybrid (26 isolates), *Phytophthora* sp. LS-2018c strain CL181 (23 isolates), *Ph. lacustris* (six isolates), *Ph. gonapodyides* (one isolate), *Ph. cactorum* (one isolate) and *Ph. cryptogea* (one isolate).

The only other study to investigate *Phytophthora* spp. from waterways in New Zealand, was carried out by Randall (2011) using a river baiting method to identify *Phytophthora* spp. present in the waterways of native forests (broad-leaf podocarp and kauri forests) in five catchment sites within the Waitakere Ranges in Auckland. Six *Phytophthora* spp. were recovered over four seasons being *Ph. multivora, Ph. gonapodyides, Ph. kernoviae, Ph. aspargi, Ph. chlamydospora* (as *Phytophthora* taxon Pgchlamydo), and a yet to be described species, *Phytophthora* sp. "Waitakere". When compared to the present study, fewer species were recovered by Randall (2011) with only four of the six *Phytophthora* spp. recovered during the summer season baiting, being *Ph. multivora* (eight isolates)*, Ph. gonapodyides* (15 isolates)*, Ph. chlamydospora* (two isolates)*, Phytophthora* sp. "Waitakere" (two isolates). Further, in the current study of two waterways, the Halswell and Liffey Rivers, the only species that was common with the study of Randall (2011) was *Ph. gonapodyides* with only one isolate recovered. The other *Phytophthora* spp. recovered differed between the two studies. *Ph. thermophila* x *Ph. amnicola* hybrid was found to be predominant in the Halswell and Liffey Rivers however, Randall (2011) did not report any hybrids being recovered in their study.

A water baiting survey in Western Australia recovered mostly clade 6 *Phytophthora* spp. diversity as seen in the current study, with two hybrids (named as hybrid A and B) and *Ph. inundata* being the predominant species in the 48 waterways studied by Hüberli *et al.* (2013). Forty-eight waterways surveyed recovered 12 *Phytophthora* spp. (*Ph. amnicola, Ph. cinnamomi* var. *pavispora, Ph. cryptogea, Ph. fluvialis, Ph. inundata, Ph. litoralis, Ph. multivora, Ph. thermophila* and four undescribed species) and two hybrids (named as hybrids A and B). Similar *Phytophthora* species diversity (nine species) to the current study were also recovered by Reeser et al. (2011) from their sampling of three streams in Western Oregon, with *Ph. gonapodyides, Ph. lacustris* (as *Phytophthora* taxon salixsoil) and *Ph. chlamydospora* (as *Phytophthora* taxon Pgchlamydo) being the most frequently recovered. The same authors also reported that of the 49 Alaskan streams *Phytophthora* spp. were only successfully recovered from 28 streams, with seven species recovered and again *Ph. gonapodyides* being the most frequent recovered (Reeser et al., 2011). Although there is some

variation in the number of *Phytophthora* species recovered from waterways this appears to be related to the sampling intensity, with more intense sampling increasing the likelihood of detection of those species infrequently recovered. One such species is *Ph. cryptogea* which was recovered at low numbers from the current study and was reported by Hüberli *et al.* (2013) to be rare in the waterways with low numbers isolated.

The difference in the species of *Phytophthora* recovered in the four studies (current survey in Canterbury, Randall (2011), Hüberli *et al.* (2013) and Reeser *et al.* (2011) could be due to the difference in land use type and regions, *Phytophthora* baits used, difference in baiting time (seasonal variation) and frequency of baiting. The current study used three *Phytophthora* baiting methods however the *Phytophthora* spp. recovered from river baiting method was similar to that recovered with the laboratory baiting and filtration method. Surveys carried out by Randall (2011), Hüberli *et al.* (2013) and Reeser et al. (2011) also used river baiting method and so it is unlikely that the difference in *Phytophthora* diversity can be attributed to the difference in baiting method. The current study in Canterbury was a small survey during summer (baiting once) in two rivers with a total of six sites having varying land use. The Liffey Stream sites were located around residential area with native planting and isolated only two *Phytophthora* hybrids. Higher *Phytophthora* diversity (six *Phytophthora* spp. including one hybrid) was observed in the Halswell River which passed through Tai Tapu village containing mixed backyard vegetation, dairy, sheep and pasture. Randall (2011) surveyed one site in each of the five different water catchments in west Auckland, however all these catchments had similar land use containing native forest (broad-leaf podocarp and kauri forests). Baiting was done once every two months for a year to cover all four seasons with seasonal variation seen in the *Phytophthora* spp. recovered and its abundance (Randall, 2011). Similarly, 48 forest streams were sampled by Reeser *et al.* (2011) in Alaska, whilst the river sites in Western Oregon varied between healthy forests and nearby agricultural and urban areas. Hüberli *et al.* (2013) study was on a larger scale involving 48 waterways in Western Australia with high variation in *Phytophthora* spp. diversity observed between different regions. Survey sites included ponds, lakes, streams and estuaries with baiting done up to four times in the same site in Spring. All the four studies carried out baiting in different seasons with different frequency. Randall (2011) reported the *Phytophthora* spp. diversity differed when the same five sites were baited in the four different seasons. Another difference in the four studies is the *Phytophthora* baits used in river baiting. The current study used leaves of *Pt. undulatum, R. arboreum, Ce. deodara, Pt. eugenioides, Pi. radiata, Ca. japonica* and *B. attenuata.* Randall (2011) used *L. angustifolius* (lupin) seedling and leaves of *A. australis* (Kauri), *Ce. atlantica* (Atlas cedar), *Pt. tenuifolium* (Kohuhu) and *R. arboreum.* Hüberli *et al.* (2013) also used *L. angustifolius* seedling and leaves of *B. attenuata, Pt. undulatum, H. laurina* (Kodjet) and *Q. robur* (English Oak) whilst Reeser *et al.* (2011) used *Chamaecyparis lawsoniana* (Lawson cypress), *Rhododendron macrophyllum, Lithocarpus densiflorus* (tanoak) and *Pyrus communus* (D'Anjou pear) for baiting the Western Oregon streams and *Rhododendron catawbiense* and *Arctostaphylos uva-ursi* (bearberry) for the Alaskan waterways. According to Hüberli *et al.* (2013) the *Phytophthora* spp.

isolated varied with the different types of plant species used as baits with some *Phytophthora* spp. being attracted to certain baits and not others.

2.4.2 Difference in *Phytophthora* diversity and land use type in Liffey Stream and Halswell River

There was no difference in *Phytophthora* spp. diversity in the four sites in the Liffey Stream which could be due to the close proximity of the sampling sites. Vegetation in the four sites were also similar where water flow was though areas planted with native trees. Low *Phytophthora* diversity (two spp.) was obtained from the Liffey Stream i.e., *Ph. thermophila x Ph. amnicola* and *Ph. amnicola x Ph. chlamydospora* hybrids. Comparatively, Halswell River had more *Phytophthora* spp. diversity (six *Phytophthora* spp.) which could be attributed to the varying land use practices surrounding the two sampling sites. Similar to the Liffey Stream, *Ph. thermophila x Ph. amnicola* hybrids were also isolated as the dominant spp. from the Halswell River sites, followed by *Phytophthora* sp. LS-2018c strain CL181, *Ph. lacustris* and *Ph. gonapodyides* in both sites. Isolation of *Ph. cactorum* and *Ph. cryptogea* from site two only could be due to the large distance (2.24 km) between the two sampling sites which contributed towards the difference in *Phytophthora* spp. diversity. Presence of *Ph. thermophila* hybrids in all the six site and *Phytophthora* sp. LS-2018c strain CL181in the Halswell River raises concern on the spread of these species as these have not previously been reported in New Zealand. More *Phytophthora* spp. are likely to be discovered if more extensive water baiting is carried out in New Zealand. Isolation of low levels (1 isolate each) of *Ph. gonapodyides, Ph. cactorum* and *Ph. cryptogea* indicates that zoospores present in lower concentration in the waterways could be missed during baiting, and repetitive water baiting needs to be done to indicate the true *Phytophthora* spp. diversity in the area.

Mainly clade 6 *Phytophthora* spp. (*Ph. amnicola, Ph. chlamydospora, Ph. gonapodyides, Ph. lacustris* and *Ph. thermophila*) and *Phytophthora* sp. LS-2018c strain CL181 belonging to clade 2b were isolated in this study. Only a low frequency of isolation of clade 1a *Ph. cactorum* and clade 8a *Ph. cryptogea* from the Halswell River was seen. Randall (2011) survey also isolated mostly clade 6 species (*Ph. asparagi, Ph. chlamydospora, Ph. gonapodyides* and *Ph. multivora*) and a clade 10 species, *Ph. kernoviae*. Similar results were also reported by Hüberli *et al.* (2013) and Reeser et al. (2011) with clade 6 species being the most frequently recovered. *Phytophthora* clade 6 species are closely associated with riparian ecosystems and frequently isolated from waterways (Hüberli *et al.*, 2013). Despite *Phytophthora* being known as devastating pathogens, closer examinations of waterways and soils have identified *Phytophthora* spp. that do not appear to be plant pathogens. Stamler *et al.* (2016a) suggests, *Phytophthora* spp. in aquatic and saturated environments are early saprophytes. It has been suggested that the clade 6 species, being the first colonisers of plant debris in waterways, enable nutrients to move up the trophic levels through zoospore grazing and creating

environmental conditions that are suitable for detritivores (Nechwatal *et al.*, 2013; Stamler *et al.*, 2016a). Sexual sterility in clade 6 is attributed to the predominant aquatic lifestyle where *Phytophthora* spp. competes with other microbes such as *Achlya, Pythium* and *Saprolegnia* to colonise the same substrate through the production of abundant asexually produced zoospores (Jung *et al.*, 2011). Dependence on asexual zoospores has led to sexual degeneration and without sexual reproduction, the clade 6 *Phytophthora* spp. have lost the ability to generate new genotypes that vary in phenotypic characteristics such as virulence, growth rates and host range (Jung *et al.*, 2011). Therefore, sexually sterile clade 6 *Phytophthora* spp. isolated from waterways are less likely to be of grave disease concern to their natural habitat. Isolation of *Phytophthora* belonging to other clades that do not depend on a predominant aquatic lifestyle shows the possible distribution of pathogenic *Phytophthora* spp. through waterways, such as *Ph. cactorum* or *Ph. cryptogea* in the current study.

2.4.3 Recovery of *Phytophthora* from Halswell River and Liffey Stream

2.4.3.1 *Phytophthora* Morphotype groups

Grouping of *Phytophthora* isolates into morphotype groups was a challenge due to the plasticity of isolates in culture. The plasticity of cultures resulted in each morphotype being further divided into subgroups, however DNA identification showed that these subgroups represented the same species. Previous water baiting studies have mostly isolated clade 6 *Phytophthora* spp. that were mainly sexually sterile with dependence on asexually produced zoospores (Stamler *et al.*, 2016a). Therefore, isolates were grouped using sporangia characteristics and colony pattern on PDA and V8A. Initially 14 day-old *Phytophthora* spp. colonies on PDA was used only, however this resulted in varying *Phytophthora* spp. being grouped under one morphotype. Thus, the colony age was reduced to 7 days and colony characteristics recorded on both PDA and V8A which gave a more refined result. For the isolates of species that were not sexually sterile i.e., *Phytophthora* sp. LS-2018c strain CL181, *Ph. cactorum* and *Ph. cryptogea;* sexual structures were not studied due to the high cost of beta-sitosterol required for production of gametangia in cultures (Jeffers, 2015b). However, despite this the morphotype grouping of isolates based on colony morphology and sporangia characteristics was sufficient to group isolates into species groups which were subsequently confirmed by DNA sequencing. This method also reduced the number of isolates required for confirmation of species identity by DNA sequencing.

2.4.4 *Phytophthora* hybrids

Two *Phytophthora* spp. hybrids, *Ph. thermophila* x *Ph. amnicola* hybrid and *Ph. amnicola x Ph. chlamydospora* hybrid, were recovered from this study, with *Ph. thermophila* x *Ph. amnicola* hybrid being the most frequently recovered *Phytophthora* spp. Similar results were reported by (Hüberli *et al.*, 2013) where of the 360 *Phytophthora* spp. isolates recovered from Western Australian waterways

112 were identified as being hybrids. In contrast, the *Phytophthora* surveys by Randall (2011) and Reeser et al (2013) did not report any isolation of hybrids. The reason for this is unclear and whether this represents a true absence of hybrids in these waterways or is due to the identification methods used to not being able to discriminate hybrid species. Randall (2011) only used the sequence result of ITS region (ITS4 and ITS6) to identify *Phytophthora* isolates recovered from waterways. Previous surveys of waterways in Australia and South Africa have also identified clade 6 hybrids to be common in waterways, with these commonly occurring between *Ph. thermophila, Ph. chlamydospora* and *Ph. amnicola* with a strong indication that these hybrids had arisen from sexual recombination due to the detection of only a single *coxI* allele in the hybrids formed (Nagel *et al.*, 2013). The present study however, did not investigate on how these hybrids were formed. The three parental spp. that were found to form hybrids i.e., *Ph. thermophila, Ph. chlamydospora* and *Ph. amnicola,* in the present study all belong to subclade II of *Phytophthora* clade 6 (Burgess & Jung, 2012; Jung *et al.*, 2011). This is in agreement with previous studies which have shown that *Phytophthora* hybrids are known to form between species of the same phylogenetic clade (Burgess, 2015). There are many reports in the literature that natural hybridization is common amongst the closely related species within clade 6 (Stamler *et al.*, 2016a). According to Jung *et al.* (2011) *Phytophthora* clade 6 has been found to have a strong association with forests and riparian ecosystem (Jung *et al.*, 2011). While many species in subclade II of clade 6 are known to have a strong association to rivers and riparian ecosystems (Nagel *et al.*, 2013).

Phytophthora thermophila x Ph. amnicola hybrid was recovered from all six sites sampled in the Liffey Stream and Halswell River, which indicates the widespread distribution of *Ph. thermophila* and *Ph. amnicola* in the Halswell and Liffey area. However, *Ph. amnicola x Ph. chlamydospora* hybrids were only isolated from the Liffey Stream, which may be due to the absence of *Ph. chlamydospora* in the Halswell area. *Phytophthora chlamydospora* has been associated with certain host plants in New Zealand and absence of these in the Halswell area could explain the absence of *Ph. amnicola x Ph. chlamydospora* hybrids from the Halswell River. *Phytophthora chlamydospora* has been previously isolated in New Zealand and has been recovered from host plants such as *Idesia polycarpa* (Chinese Wonder Tree)*, Liquidambar styraciflua* (American Liquidambar) and *Macropiper excelsum* (Kawakawa) in Northland, Auckland and Taranaki (Manaaki Whenua Landcare Research, 2019d). Association of *Ph. chlamydospora* to exotic trees such as *I. polycarpa* and *L. styraciflua* and the native tree *M. excelsum* in New Zealand indicates the possible pathogenicity of the species to native and exotic forests and possible implications of *Ph. chlamydospora* hybrids forming in waterways that could lead to disease outbreaks due to anthropogenic activities. *Phytophthora chlamydospora* has been commonly isolated from waterways and riverbank soil around the world (Aghighi *et al.*, 2016). Frequent isolations from irrigation waters, streams, rivers and riparian soils in forests in Europe, Asia, Africa and in western North and South America have been noted (Hansen *et al.*, 2015). In Australia, *Ph. chlamydospora* has been isolated from soils of native forests and streams (Jung *et al.*, 2011). *Phytophthora chlamydospora* is known to be an opportunistic and sometimes aggressive pathogen of

trees (Aghighi *et al.*, 2016). Although the current study did not isolate the *Ph. amnicola, Ph. chlamydospora* and *Ph. thermophila* strains, these species were isolated as parents of the hybrids which means that it is present in the environment possibly as a pathogen.

Isolation of *Ph. amnicola* hybrids from the Liffey Stream and Halswell River is supported by the previous isolation of *Ph. amnicola* in New Zealand. Isolates of *Ph. amnicola* have been previously recovered from river baiting surveys in Auckland by Randall (2011) and recorded in the New Zealand Fungi website (https://nzfungi2.landcareresearch.co.nz). Apart from this, *Agathis australis* (Kauri) which is a native tree of significant Maori value has also been recorded as a host for *Ph. amnicola* (Manaaki Whenua Landcare Research, 2019d). Although *Ph. amnicola x Ph. chlamydospora* hybrid has been previously isolated in New Zealand there are no records of *Ph. thermophila* x *Ph. amnicola* hybrid (Manaaki Whenua Landcare Research, 2019d). Reports on the detection of *Ph. amnicola* from other countries is limited. *Phytophthora amnicola* and *Ph. thermophila* x *Ph. amnicola* hybrid was described as novel spp. isolated from waterways in Australia with a possibility of these spp. being of Australian origin due to their frequent recovery (Nagel *et al.*, 2013). In Western Australia, *Ph. amnicola* was isolated from both a host plant, *Patersonia* sp., and stream baiting, while *Ph. thermophila* x *Ph. amnicola* hybrids were isolated from soils of native forest and stream baiting. Although it has been suggested that *Ph. amnicola* is Australian in origin the frequent recovery of *Ph. amnicola* and *Ph. amnicola* hybrids in the two NZ water surveys suggest that it is widespread in New Zealand and requires further study into its potential impact on native and crops

Isolation of *Ph. thermophila* hybrid isolates from both the Liffey Stream and Halswell River is a new finding as *Ph. thermophila* has not been reported from New Zealand, and although it was not recovered in the present study, the presence of the isolates identified as *Ph. thermophila x Ph. amnicola* hybrids indicates that the parent strain is present. The frequent isolation of *Ph. thermophila* hybrids indicates that *Ph. thermophila* is well established in the natural ecosystem in Canterbury. Since Randall (2011) did not recover any *Ph. thermophila* from the Auckland survey, it is more likely that this species is established in Canterbury around the Liffey Stream and the Halswell River and not distributed widely across New Zealand. However, further work is needed to confirm its distribution. In other studies, *Ph. thermophila* has only been reported from Australia with its isolation from stream baiting, soils of four dying native plant species (*Eucalyptus marginata*, *Bankasia grandis, Xanthorrhoea gracilis* and *Xanthorrhoea preissii*), several plant species belonging to the genera *Grevillea, Eucalptus, Hakea* and *Patersonia* and exotic timber species including *Pi. radiata* (Jung *et al.*, 2011). According to Aghighi *et al.* (2016), *Ph. thermophila* are opportunistic pathogens under episodic conditions like flooding Disease expression usually tend to be low impact with few scattered plant deaths (Jung *et al.*, 2011) and therefore its presence and those of the hybrids in Canterbury waterways may not be a major issue under normal environmental conditions to native and crop plants.

While *Ph. thermophila, Ph. chlamydospora* and *Ph. amnicola* have been identified as being present in New Zealand, Nagel *et al.* (2013) suggests the origin of these three species to be Australia with

subsequent introduction into South Africa. Further surveys in New Zealand is required to identify the current spread of these species which could confirm the possibility of these being introduced species to New Zealand or potentially part of the native microbiota.

2.4.5 *Phytophthora* sp. LS-2018c strain CL181

Phytophthora sp. LS-2018c strain CL181 is a new species recorded to New Zealand isolated only from the two sites in the Halswell River. Previously isolated by Jung *et al.* (2018a) and given the provisional name *Ph. valdiviana,* this is a new species isolated from streams of Valdivian forest in Chile and believed to be a native to the Valdivian region of Chile. Grouped in phylogenetic clade 2b, *Phytophthora* sp. LS-2018c strain CL181 closely resembles its close relative *Ph. siskiyouensis* (Jung *et al.*, 2018a). Isolates identified as *Phytophthora* sp. LS-2018c strain CL181based on sequencing of the *coxI* gene (99% to 100% sequence identity) in this study, were initially believed to be *Ph. siskiyouensis* based on ITS (99% sequence identity). However, morphological difference between *Phytophthora* sp. LS-2018c strain CL181 and *Ph. siskiyouensis* exists. *Phytophthora siskiyouensis* isolates tend to have either amphigynous or paragynous antheridia while *Phytophthora* sp. LS-2018c strain CL181 exclusively have amphigynous antheridia (Jung *et al.*, 2018a; Reeser *et al.*, 2007). However, sexual structures were not examined in this study. Neither *Ph. siskiyouensis* nor *Phytophthora* sp. LS-2018c strain CL181 have been recorded in New Zealand and are likely to be a new species to New Zealand. Current identification of these isolates was accepted as *Phytophthora* sp. LS-2018c strain CL181 as identified on *coxI* with a 100% match to Jung *et al.* (2018a) *coxI* sequence in GenBank. However, for confirmation of the isolates identity further work will be needed, including production and examination of the sexual structures. According to Jung *et al.* (2018a), *Phytophthora* sp. LS-2018c strain CL181 has proven to be pathogenic to *Quercus suber* (Cork Oak) found in Valdivian rainforest. *Quercus suber* is an introduced species in New Zealand (Thorpe, 2017) and a possible host for *Phytophthora* sp. LS-2018c strain CL181. More research is required to investigate the introduction and spread of *Phytophthora* sp. LS-2018c strain CL181in New Zealand.

2.4.6 *Phytopthora lacustris*

Phytophthora lacustris was isolated from all the sites in both the Liffey Stream and Halswell River, which indicates it is widespread in the two areas. Previously known as *Phytophthora* taxon Salixsoil, a native to the United Kingdom, *Ph. lacustris* was first isolated from *Salix matsudana* (Chinese willow) roots in 1972 (Nechwatal *et al.*, 2013; Stamler *et al.*, 2016a). With a global distribution, except for the tropics, *Ph. lacustris* is now widely detected in Europe, Australia, New Zealand and the USA from wetlands, aquatic habitats and nurseries (Nechwatal *et al.*, 2013; Stamler *et al.*, 2016a). *Phytophthora lacustris* invades the fine roots of trees that are under stress due to flooding or drought and is a mild pathogen on *Prunus* spp., *Salix* spp. and *Alnus* spp. (Nechwatal *et al.*, 2013; Stamler *et al.*, 2016a).

According to Nechwatal *et al.* (2013), *Ph. lacustris* has been found in the nursery trade in Europe and causes root rots in commercial fruit trees. *Phytophthora lacustris* has been previously isolated in Auckland and believed to be established in New Zealand (Manaaki Whenua Landcare Research, 2019d). However, little is known on the association of *Ph. lacustris* to *Prunus* spp., *Salix* spp. and *Alnus* spp. that are present in New Zealand. Despite being exotic, *Ph. lacustris* appears to have co-evolved in the riparian habitat without report of any disease outbreaks.

2.4.7 *Phytophthora gonapodyides*

Only one isolate of *Ph. gonapodyides* was recovered from site one in Halswell River indicating low levels of zoospores in the waterways during baiting. However, *Ph. gonapodyides* is an established species in New Zealand with previous association with exotic trees and fruit trees of economic importance. *Phytophthora gonapodyides* has been isolated from *Castanea sativa* (Sweet chestnut) roots in South Canterbury, *P. eugenioides* (lemonwood) in mid Canterbury, soils around *Quercus robur* (English Oak) roots in Auckland, *Actinidia deliciosa* (Kiwifruit) and *Juglans regia* (English Walnut) trees (Manaaki Whenua Landcare Research, 2019a; Stewart & McCarrison, 1991). With a strong association to forest and riparian ecosystem, *Ph. gonapodyides* also has association to agriculture, horticulture (Jung *et al.*, 2011) and known to be an opportunistic and sometimes aggressive pathogen of trees (Aghighi *et al.*, 2016; Nechwatal *et al.*, 2013). With a wide host range, *Ph. gonapodyides* has been found to be pathogenic on various trees in Australia, the USA, Denmark and Europe (Ristaino, 2019). In Southern Sweden, *Ph. gonapodyides* causes stem canker on *Fagus sylvatica* (European Beech). Climatic triggers such as high precipitation and mild winter temperatures in Sweden have provided favourable conditions for *Ph. gonapodyides* to spread, with old beech stands being more susceptible favouring stem canker disease (Cleary *et al.*, 2016). Due to its wide host range, *Ph. gonapodyides* is likely to be wide spread in New Zealand. Further research on the potential pathogenicity of *Ph. gonapodyides* under climate change conditions is needed to identify the possible implications of this pathogen on New Zealand's natural ecosystem.

2.4.8 *Phytophthora cactorum*

Phytophthora cactorum (1 isolate) was isolated in the Halswell River site 2 and is a pathogenic species known to be established in New Zealand (Manaaki Whenua Landcare Research, 2019e). *Phytophthora cactorum* a homothallic clade 1a species, initially detected in the 1870s, has a wide host range affecting over 250 plant species in 150 genera worldwide (Hudler, 2013, 2019). In forest trees, *Ph. cactorum* has been isolated from dying seedlings of forest trees such as *Abies* spp., *Acacia* spp., *Acer* spp., *Cedrus* spp., *Fagus* spp., *Larix* spp., *Picea* spp., *Pinus* spp., and *Robinia* spp. (Hudler, 2013). It also causes disease in commercial roseaceous fruit trees (Hudler, 2013). Commonly known to cause crown and collar rots on seedlings, strains of *Ph. cactorum* also cause serious diseases such as lethal

root collar cankers on *Acer* spp., *Abies* spp.,and *Cornus* spp. (Hudler, 2013). The Tai Tapu area, through which Halswell River site two passes, does not have any commercial farms growing possible *Ph. cactorum* host spp. Therefore, individual host plants such as *Diospyros kaki* (Persimmon), *Acca sellowiana* (Feijoa), *Potentilla* (cinquefoil), *Malus domestica*, (Apple), *Pyrus communis* (Pear), *Alnus cordata* (Alder) and the endemic tree *Vitex lucens* (Puriri) (Manaaki Whenua Landcare Research, 2019e) could be the source of *Ph. cactorum* isolated. *Phytophthora cactorum,* which is commonly isolated via soil baiting and diseased plant materials, has the ability to spread through irrigation water (Rivero *et al.*, 2011). Isolation of *Ph. cactorum* from the river raises concern of the possible transfer of the pathogen via waterways and the likely implications this could have on irrigation of orchards that is done using river water. Further research is required to determine the presence of this pathogen in waterways associated with orchards in New Zealand.

2.4.9 *Phytophthora cryptogea*

Phytophthora cryptogea (1 isolate) was isolated from Halswell River site 2, which indicates that this pathogen is present in low levels and is possibly associated with a diseased host found only in its proximity. Widespread in New Zealand *Ph. cryptogea* is a heterothallic species belonging to clade 8a which has been found in association with *A. deliciosa* (Kiwifruit), *Solanum lycopersicum* (Tomato), *Solanum melongena* (Eggplant), *Prunus persica* (Peach), *Agathis australis* (Kauri), *Corokia* spp., *Podocarpus totara* (Torara), *Spinacia oleracea* (Spinach) and *Xanthorrhoea australis* (Grass tree) (Manaaki Whenua Landcare Research, 2019c). *Phytophthora cryptogea* has a worldwide distribution with a wide host range (Brasier *et al.*, 2003; Safaiefarahani *et al.*, 2015) and has previous associations with *Pi. radiata* death (Bumbieris, 1976), foot-rot disease in fruit trees and horticultural crops and root rot in the hedge plants such as *Syzygium smithii* (Acmena), *Melaleuca* spp., *Matthiola incana* (hoary stock), *Tagetes erecta* (marigold) and other ornamental plants (CABI, 2019; Safaiefarahani *et al.*, 2015). *Phytophthora cryptogea* has been reported to be pathogenic in forests when trees are subjected to intermittent waterlogging (Aghighi *et al.*, 2016). Isolation of low levels of *Ph. cryptogea* in Halswell River site 2 could possibly be due to a diseased host plant in a nearby garden or shelter belt.

2.4.10 Evaluation of *Phytophthora* recovery methods

The number of *Phytophthora* isolates and *Phytophthora* spp. (diversity) recovered by the river baiting and the laboratory baiting methods were similar with a slight difference in the *Phytophthora* spp. diversity. For the Halswell river sites this difference appears to be mainly due to the relative recovery of infrequently isolated species along with the commonly isolated species and hybrid, with one isolate of *Ph. gonapodyides* isolated in the laboratory baiting, and one isolate of each *Ph. cactorum* and *Ph. cryptogea* by the river baiting. In contrast, the filtration method recovered the lowest number of *Phytophthora* isolates and *Phytophthora* spp., with only two species recovered. Recovery of *Ph.*

cactorum and *Ph. cryptogea* using the river baiting only method indicates that zoospores of these species were present in low concentrations or occurred periodically in the waterways and these species could not be captured during collection of water samples. Currently, the river baiting method is the most widely used method for *Phytophthora* recovery from ponds, rivers, lakes and streams (APHIS, 2014). The recovery of *Phytophthora* spp. by river baiting indicates that the zoospores of these species were present in high levels during the baiting period resulting in the encysting of the zoospores on the leaf baits causing infection (Randall, 2011). In the present study, the similar success in the recovery of *Phytophthora* species in the laboratory baiting indicate that there were sufficient zoospores in the water samples to result in infection of the leaf baits.

Laboratory baiting recovered the three *Phytophthora* spp. from Halswell River that was frequently isolated in river baiting i.e., *Ph. thermophila x P. amnicola* hybrids (21 isolates), *Phytophthora* sp. LS-2018c strain CL 181(three isolates) and *Ph. lacustris* (two isolates). In addition, laboratory baiting isolated one isolate of *Ph. gonapodyides*. *Phytophthora gonapodyides* was morphologically grouped with *Ph. thermophila x Ph. amnicola* hybrids in morphotype group M3a due to the morphological similarity. Therefore, *Ph. gonapodyides* isolates that may have been isolated using the river baiting method could have been misidentified as hybrids. Laboratory baiting (also known as bottle of baits) is popular for *Phytophthora* recovery in intermittent streams, forests and nurseries, and is effectively used for *Ph. ramorum* surveillance in USA (APHIS, 2014; Ivors, 2018; Parke & Rizzo, 2011) and the results of this study indicate that it could be used to survey New Zealand waterways to determine *Phytophthora* spp. diversity.

The filtration method only recovered two *Phytophthora* spp., *Phytophthora* sp. LS-2018c strain CL181 (four isolates) and one *Ph. lacustris* isolate. This is because the majority of *Phytophthora* colonies were overgrown by *Pythium* spp. which hindered with *Phytophthora* isolation. In the baiting method, *Pythium* spp. are controlled using surface sterilization of leaf baits, however in the filtration method the membrane filters recovered the different microbes present in the waterways. Hymexazol was used to suppress *Pythium* growth however, this only works on certain *Pythium* spp. Although, hymexazol inhibits most *Pythium* and *Morteriella* spp. that overgrow *Phytophthora* colonies (Martin *et al.*, 2012), several *Pythium* spp. such as *Py. irregulare* and *Py. vexans* (reclassified *Phytopythium vexans*; de (de Cock *et al.*, 2015)) are resistant (Drenth & Sendall, 2001). The results indicate that these hymexazol resistant *Pythium* or *Phytopythium* species maybe abundant in these two Canterbury waterways. The poor *Phytophthora* recovery using the filtration method in this study could also be attributed to the lack of replication in filtering water samples for each site. Hwang *et al.* (2008) identified the filtration method to be a more effective and efficient method than the baiting method for detecting *Phytophthora* spp. diversity, where *Phytophthora* isolation was done using the same membrane filters as used in the current study, however nine replicates 100 mL water samples were filtered from the 1L water sample collected for each site.

The three *Phytophthora* isolation methods i.e., river baiting, laboratory baiting and filtration method, have advantages and disadvantages. An advantage of the river baiting is that the *Phytophthora* recovery represents the *Phytophthora* population over time due to longer exposure of the baits and baits can be stored longer before processing (Ivors, 2018). While laboratory baiting and the filtration method only represent the *Phytophthora* population at the time of sampling (Ivors, 2018). Therefore, river baiting is more likely to isolate *Phytophthora* spp. inoculum present in lower concentration or periodically in waterways. *Phytophthora* recovery by river baiting is affected by exposure time of leaf baits, water temperature and leaf material; colonisation of leaves by other microbes; loss of baits after major rain; and missing baits due to public curiosity (Ivors, 2018). While in laboratory baiting and filtration, all water samples are processed at the same room temperature and loss of bait bags or missing baits due to public curiosity is reduced. Fewer trips to the sampling sites for water collection are required than river baiting however, water samples needs to be processed within 10 hrs of collection which can be an issue if the sample sites are some distance from the laboratory for processing (Hwang *et al.*, 2008; Ivors, 2018). Collecting water samples immediately after rain can cause diluted zoospore inoculum and turbid water which affects results (Ivors, 2018). *Phytophthora* spp. recovery using laboratory baiting, can be improved by multiple water sampling collected from a site over time to provide a true representation of *Phytophthora* spp. diversity in an area. The filtration method is reported to provide greater *Phytophthora* spp. diversity than baiting method and captures slow growing *Phytophthora* that have specific growth requirements (Dunstan *et al.*, 2016). Apart from this, the filtration method allows *Phytophthora* inoculum in waterways to be quantified (Bush *et al.*, 2003). *Phytophthora* recovery using multiple methods and using a range of baits at multiple locations over different seasons is strongly recommended by Dunstan *et al.* (2016) for determining the true representation of the *Phytophthora* spp. diversity. For *Phytophthora* spp. isolated in lower numbers use of additional isolation methods and repeated sampling across different seasons can increase the number of positive isolations with a possibility of isolating new *Phytophthora* spp. (Huai *et al.*, 2013).

2.4.11 Evaluation of leaf baits for baiting for *Phytophthora* spp.

Regardless of the baiting method, *R. arboreum* was identified as a preferable bait for recovering larger numbers of *Phytophthora* isolates compared with the other six leaf baits, while *Ca. japonica* did not recover any *Phytophthora.* Whilst there was no statistical difference for the laboratory baiting, both the river baiting and combined analysis showed significantly higher recovery from the *R. arboreum* baits. As discussed earlier the baits floated in the river are exposed to higher relative zoospore inoculum levels over a longer period of time which probably results in higher levels of infection, especially for the more susceptible bait such as *R. arboreum*. Further, observations on the species recovered on the different bait types also showed that some baits recovered certain *Phytophthora* spp. which others did not. This finding is in line with Hüberli *et al.* (2013) who found that the *Phytophthora* spp. isolated varied with the different types of plant species used as baits and some

Phytophthora spp. were attracted to certain baits and not others. The current study identified *Pt. undulatum* as a good bait that targeted the highest number (four species) of *Phytophthora* spp. *Pittosporum undulatum* isolated the frequently found *Ph. lacustris* and *Ph. thermophila x amnicola* hybrids; and isolated the rarer *Ph. cactorum* and *Ph. gonapodyides*. *Rhododendron arboreum* was also a good bait as it recovered the frequently occuring *Phytophthora* sp. LS 2018c strain CL 181, that was not obtained from *Pt. undulatum.* Apart from this *R. arboreum* also recovered the rarely occurring *Ph. cryptogea* (not isolated from *Pt. undulatum)* and the frequently occurring *Ph. thermophila x amnicola* hybrids. The other four baits isolated the more commonly occurring *Phytophthora* spp. A combination of *Pt. undulatum, R. arboreum*, *P. radiata* and *Ce. deodara* could be used effectively for studying *Phytophthora* diversity. This is supported by Dunstan *et al.* (2016) who stated that no single bait can be used to isolate all *Phytophthora* spp., and although the species recovered is not dependent on the type of bait used, bait type does affect the number of *Phytophthora* isolations. Martin *et al.* (2012) reported leaves of *Rhododendron* spp. yield greater diversity of *Phytophthora* spp. and populations. Surveillance of waterways for *Phytophthora* spp. in Australia has used *Pt. undulatum*, *Quercus robur, Hakea laurina, B. attenuate* leaves and germinated *Lupinus angustifolius* (lupin) seedlings (Hüberli *et al.*, 2013). Most *Phytophthora* spp. were isolated from *Pt. undulatum* and *B. attenuate* and this is because they did not degrade while the lupin seedlings and *Q. robur* leaves often decomposed (Hüberli *et al.*, 2013). Randall (2011) found that *L. angustifolius* seedlings, *Ce. deodara* needles and *A. australis* leaves resulted in poor isolation of *Phytophthora* spp. with most isolates obtained from *R. arboreum* leaf midrib and *Pt. tenuifolium* (kohuhu)

2.4.12 *Phytophthora* Isolation media

Since the filtration method was not selected for further *Phytophthora* isolation, use of hymexazol in the *Phytophthora* isolation media was not necessary. Further since some *Phytophthora* spp. are sensitive to hymexazol (Martin *et al.*, 2012) such as *Ph. cactorum, Ph. palmivora, Ph. lateralis, Ph. cinnamomi* and *Ph. citrophthora,* while *Ph. infestans* is completely intolerant (Drenth & Sendall, 2001; Jeffers & Martin 1986) P_5ARP CMA was found to be adequate for *Phytophthora* isolation from the baiting method.

2.5 Conclusions

This study has identified two *Phytophthora* spp. (*Phytophthora* sp. LS-2018c strain CL181 and *Ph. thermophila* hybrids) that have not been previously reported in New Zealand. *Phytophthora* isolation in New Zealand has been undertaken mainly using soil baiting and direct isolation from diseased plants (Manaaki Whenua Landcare Research, 2019d). However, this and other studies have shown that detection of new *Phytophthora* spp. via river baiting to be successful, prompting a need for more baiting of waterways for *Phytophthora* to be carried out. The current list of *Phytophthora* spp. present

in New Zealand is likely not to be a true representation of *Phytophthora* diversity in New Zealand as waterways surveillance for isolation of *Phytophthora* spp. has widely been left unexplored. Results of water baiting done in Auckland and now in Canterbury have both isolated different and new *Phytophthora* spp. All the *Phytophthora* spp. isolated in this study are reported to be exotic species with their introduction into New Zealand not known (Scott & Williams, 2014). These species have co-evolved in the new environments without causing major disease outbreaks. It is important to carry out a more extensive study to identify the presence of *Phytophthora* spp. that pose a threat to agriculture, forestry and natural ecosystem.

This study mostly recovered the clade 6 spp. (*Ph. lacustris, Ph. gonapodyides, Ph. thermophila, Ph. chlamydospora, Ph. amnicola*), which is similar to the results of other *Phytophthora* studies in waterways. However, isolation of species in waterways is not limited to clade 6 with *Phytophthora* sp. LS-2018c strain CL181 belonging to clade 2b, *Ph. cactorum* from clade 1a and *Ph. cryptogea* from clade 8a also recovered in this study. Due to their saprophytic nature, clade 6 species are likely to continue co-evolving in their current natural habitat without causing disease outbreaks. Due to sexual sterility, clade 6 species have lost their ability to generate new genotypes that could have vary in phenotypic characteristics such as virulence, growth rates and wider host range. Based on this, clade 6 species are indicated to pose a lower threat to the natural ecosystem. However, anthropogenic activities and climate change is likely to pose a threat of altering the current host pathogen-pathogen relationship. Environmental conditions that are more favourable for pathogen development, while stressing out the host is likely to cause disease developments to be more severe.

This study identified water baiting, either laboratory baiting and river baiting as being suitable to study of *Phytophthora* spp. diversity in waterways. As no single bait can be used to isolate all *Phytophthora* spp., this study identified using combination of *Pt. undulatum, R. arboreum*, *Pi radiata* and *Ce. deodara* to be effective for studying *Phytophthora* spp. diversity. Use of *Phytophthora* recovery method is dependent on the varying needs that arises from the factors discussed and *Phytophthora* recovery using multiple a range of baits at multiple locations is strongly recommended by Dunstan *et al.* (2016) for an assessment of the true representation of *Phytophthora* spp. diversity. A more extensive *Phytophthora* spp. diversity survey in Canterbury waterways (Chapter 3) will be carried out using laboratory baiting using *R. arboreum*, *Pi radiata* and *Ce. deodara.*

Chapter 3: Identification of *Phytophthora* species diversity in Canterbury waterways

3.1 Introduction

Various abiotic factors have been found to drive fungal and oomycete populations. Climate and soil resources have been known to play an important role in fungal distribution, with strong variation in fungal colonization within a host plant (Ranelli *et al.*, 2015). Fungal colonization was found to decline with elevation, geography (longitude and latitude) and edaphic (soil) factors (Ranelli *et al.*, 2015). Whilst the Ranelli *et al.* (2015) study identified a strong association between host specificity and fungal colonisation, other prior studies have shown that different fungal groups do not share the same drivers. Geography (longitude and latitude), precipitation, temperature, soil clay content, and soil electrical conductivity were also found to affect the oomycete distribution in a study conducted in soybean fields (Rojas *et al.*, 2017). Previous research has mainly focused on abiotic factors affecting distribution and pathogenicity of oomycetes such as *Phytophthora* in soil and terrestrial plants. The continual isolation of *Phytophthora* spp. from waterways in numerous studies have led to the evaluation of the abiotic factors that influence the diversity of various *Phytophthora* spp. The relationship between the environmental drivers of aquatic and terrestrial *Phytophthora* spp. has been evaluated by Redondo *et al.* (2018). Similar to Ranelli *et al.* (2015) findings on fungal groups, Redondo *et al.* (2018) suggest that not all abiotic factors have the same impact on different groups (clades) of *Phytophthora* spp. Redondo *et al.* (2018) compared the effect of abiotic factors on *Phytophthora* spp. isolated from soil in different land use types and the waterways of the same site. For terrestrial *Phytophthora* spp., there was a decline in *Phytophthora* diversity and functional diversity (physiological traits) when temperature and precipitation decreased, with precipitation levels having a stronger impact than temperature (Redondo *et al.*, 2018). The impact of temperature and precipitation on aquatic *Phytophthora* spp. was opposite to that in terrestrial species. In aquatic species, temperature and water chemistry (pH, conductivity, total nitrogen, and total organic carbon) were the strongest drivers with *Phytophthora* spp. diversity increasing with decreasing precipitation and temperature. The difference in impact of temperature and precipitation on terrestrial and aquatic *Phytophthora* spp. was linked to the difference in buffering of these two factors by water and soil. Environmental filtering is an ecological process where the environment selects for functional traits determining the species diversity in an area (Redondo *et al.*, 2018). The effect of environmental filtering was higher on terrestrial (20%) *Phytophthora* spp. than on aquatic (3%) species (Redondo *et al.*, 2018).

This chapter aims to identify the diversity of *Phytophthora* spp. in 25 sites with varying land use types along six Canterbury waterways. The effect of abiotic factors (water parameters) such as water

temperature, pH, nitrogen and salinity on *Phytophthora* spp. diversity was evaluated. The hypothesis of this study is that *Phytophthora* diversity is affected by land use type and water parameters.

3.2 Materials and Method

Baiting was done in autumn (May 2018) to observe the variation in *Phytophthora* spp. from different waterways using the most effective bait and baiting method determined in Objective 1 (Chapter 2). Three 1L water samples were collected from a total of 25 sites in the six waterways i.e., Selwyn River (Waikirikiri), Ashburton River (Hakatere), Prices Valley River, Kaituna Valley River, Halswell River and Lake Hood. Ashburton River and Selwyn River were the two major rivers sampled with six and eight sites, respectively. The rivers were followed from the Canterbury foothills, out towards the sea with water samples taken at different sites representing different types of land use. Two rivers in Banks Peninsula, being the Kaituna Valley River (three sites) and Prices Valley River (four sites), were also sampled. Sampling of three sites in Halswell River and one site in Lake Hood, lying in the Canterbury plains, was also included in the study. Land use type, surrounding vegetation, water temperature and GPS coordinates (Appendix A.3.1, Table A1) were recorded for each site.

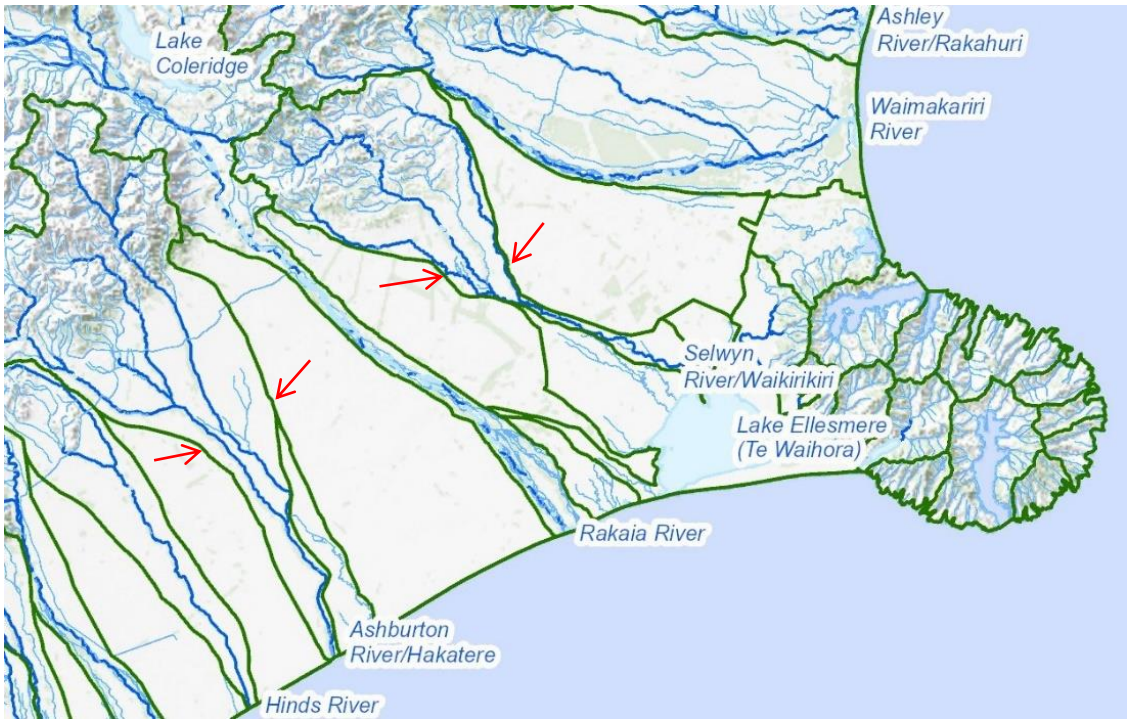

Figure 3.1: Waterway systems sampled to determine the presence of *Phytophthora* in Canterbury. The two major rivers, the Ashburton River and the Selwyn River were sampled with the blue line indicating the river while the green line (marked with red arrows) is the water catchment boundary for each river. Source: Land Information New Zealand (2019).

Water samples were collected from each site in clean 1 L plastic bottles and water temperature recorded as described in Section 2.2.1.1. The water sample was labelled and kept cool in a chilly bin during the transport back to the Lincoln University laboratory. The laboratory baiting set up was done on the same day as collection. Laboratory baiting was done at room temperature as described in Section 2.2.1.2 using two of each leaf baits of *R. arboreum, Pi. radiata* and *Ce. deodara* per water sample. Three replicates for each site (one for each of the three x 1L water samples collected per site) was set up. Baits were harvested after 7 days and *Phytophthora* isolation was done as described in Section 2.2.2. The morphotype groups in Section 2.2.5 were used to group the isolates and 10% to 20% of isolates were randomly selected from each group for DNA identification (Section 2.2.6).

Possible hybrids (non-sporangia forming isolates that produced bands of 1200 bp with amplification using the ITS primers) were identified using sequencing of the β-tubulin gene (also referred at the TUBB gene), using primers TUBUF2 (5′ CGGTAACAACTGGGCCAAGG 3') and TUBUR1 (5' CCTGGTACTGCTGGTACTCAG 3'; Section 2.2.6.2; Table 2.1). Forward and reverse sequences respectively were aligned and used to confirm the *Phytophthora* species. The PCR reactions was performed with a final volume of 20 µL which contained, 1 µL of TUBUF2 and TUBUR1primer (final concentration 0.5µM); 10 µL (1 unit) Dream *Taq* DNA polymerase (Thermo Scientific™) which includes Dream *Taq* DNA Polymerase, 2X Dream *Taq* Green buffer, dNTPs, and 4 mM $MgCl_2$ and 2 µL (approximately 20 ng) of DNA, a negative control (no DNA) was also included in each PCR run. The PCR reaction included one cycle of initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 30 secs, annealing at 60°C for 30 secs, and extension at 72°C for 1 min; a final extension at 72°C for 10 min (Appendix A.3.; Table A2). The resulting product was stored at -20°C until used.

The *Phytophthora* spp. isolates were all stored as mycelium colonised agar plugs in water as described in Section 2.2.4.

3.2.1 Nitrogen, water salinity, and pH testing

The pH, ammonium and nitrate nitrogen levels and water salinity of the water sample from each sampling site was determined. For water nitrogen and salinity tests, 30 mL of a bulked water (three water samples collected at each site was mixed) sample from each sampling site was placed in a Universal glass bottle and kept frozen (-20°C) until samples were processed at the Lincoln University Soil Science Department Analytical services. Two forms of nitrogen (mg/L) in water was measured. Ammonium (NH_4) and nitrate (NO^3) using the Flow Injection Analysis (FIA) method. Since sample site varied from their distance to the river mouth, the potential effect of salinity levels on *Phytophthora* community structure was also determined. The same water sample was used to determine salinity (parts per thousand (ppt)), level which was done using a conductivity method that measures the level of salts present in the water.

The pH measurement was done within 24 hr of water collection. Universal glass bottle filled with 30 mL of the bulked water sample from each site was taken for pH reading. The pH was taken using a pH meter (S2K712, ISFETCOM, Japan) calibrated to pH 7.0.

3.2.2 Data analysis

All data analysis was performed in R (v 3.4.3) using the platform R Studio (v 1.1.419) and Genstat for Windows 19th Edition (VSN International 2017). Differences amongst the 25 river sites were evaluated based on the number of *Phytophthora* spp. and number of *Phytophthora* isolates recovered. Analysis was also done by including data from all sites and by excluding sites and bait types that did not recover any *Phytophthora.* Analysis of variance (one-way and two-way ANOVA) was used to identify if there was a significant difference (P=0.05), followed by multiple comparisons of means using Tukey's honestly significant difference (HSD) test to identify which sites were significantly different at P=0.1.

The relationship between the number of *Phytophthora* spp. and number of *Phytophthora* isolates recovered from the 25 sites was analysed against the five abiotic factors (water temperature, pH, salinity, ammonium nitrogen and nitrate nitrogen) using Pearson's correlation. For each relationship the P-value (P= 0.05) was obtained to identify the level of significance for each correlation.

Phytophthora recovery in two seasons, summer and autumn, were analysed from Halswell River site 1 and 2 for the number of *Phytophthora* spp., number of *Phytophthora* isolates and the five abiotic factors (water temperature, pH, salinity, ammonium nitrogen and nitrate nitrogen) using a two-way ANOVA.

The three leaf baits (*R. arboreum, Pi. radiata* and *Ce. deodara*) was analysed based on the number of *Phytophthora* isolates and number of *Phytophthora* spp. recovered from each baiting type. Analysis of Variance was used to identify if there was a significant difference (P=0.05) followed by Tukey's HSD test (P= 0.05) and Bonferroni test to identify which bait type was best.

3.3 Results

3.3.1 *Phytophthora* morphotypes

A total of 265 isolates were obtained from the 25 sites sampled (Appendix A.3.1) and baited in the laboratory (Figure 3.2). Isolates preliminary identified as *Phytophthora* spp. (137 isolates) based on colony morphology were separated from *Pythium* spp. as described in Section 2.3.2 and grouped into morphotype groups based on morphology of 7 day-old colonies on PDA and V8A and sporangia morphology. The morphotype group identified in Chapter 2 was also used for grouping isolates in this current chapter (Chapter 3).

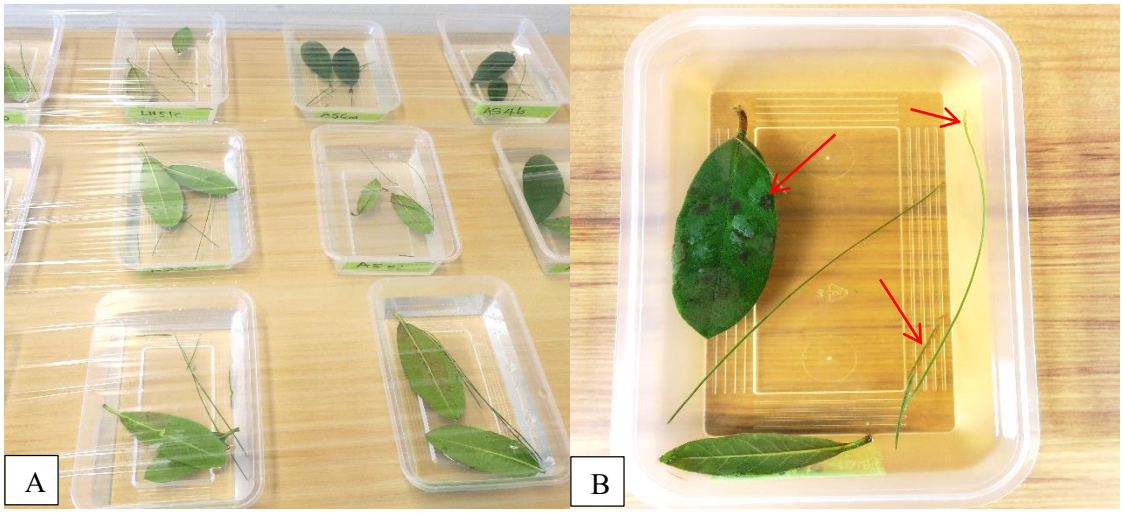

Figure 3.2: Laboratory baiting; (A) experimental set up with two of each leaf bait type i.e. *R. arboreum*, *Pi. radiata* and *Ce. deodara* placed in the water samples and incubated on a laboratory bench at room temperature, (B) red arrows showing lesion areas which developed on the leaf baits

Morphotype M2

Twenty-two isolates were identified in subgroup M2a having fast growing uniform colonies after 7 days growth on PDA and V8A. Isolates in subgroup M2a were identified as *Pythium* spp. as described in Section 2.3.2 and were discarded from further identification. No isolates were identified as belonging to subgroup M2b (Section 2.3.2). Ninety-three isolates with fast growing uniform colonies after 14 days growth on PDA (Figure 3.3), but which did not produce sporangia in sterile water, sterile soil extract solution (1%) and non-sterile soil extract solution (1%) were placed in sub group M2c. After 7 days growth on PDA and V8A, the isolates still produced fast growing and uniform colonies (Figure 3.4).

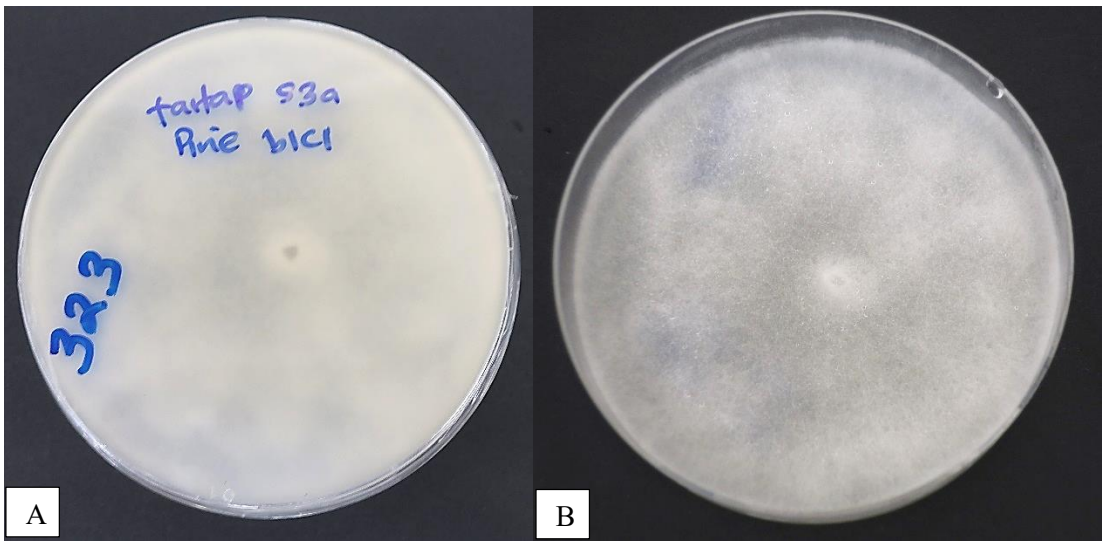

Figure 3.3: Morphotype M2c, 14 day-old uniform colony on PDA; (A) bottom view and (B) top view.

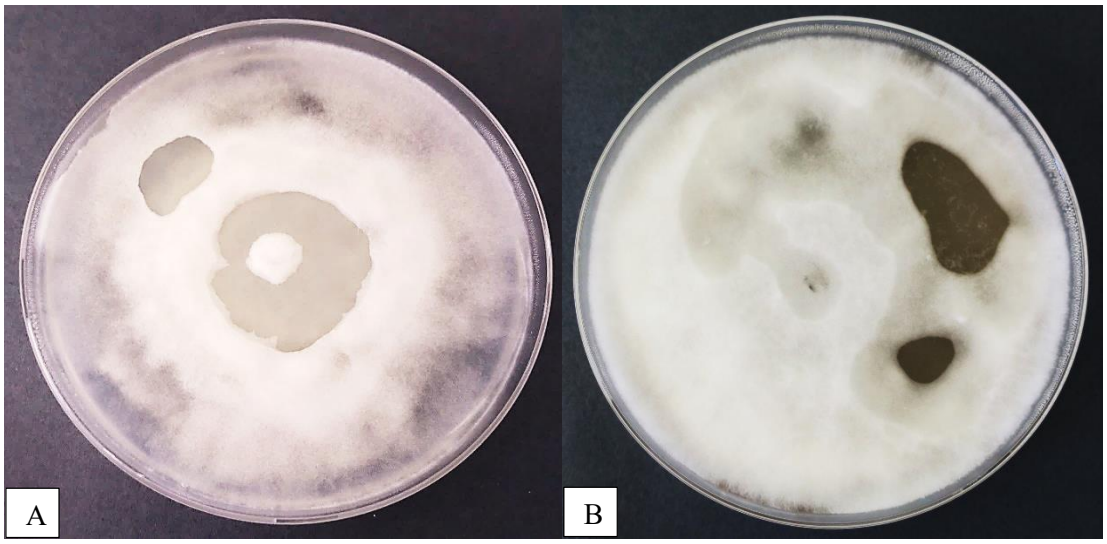

Figure 3.4: Morphotype M2c, 7 day-old colony; (A) fast growing uniform colony on PDA, and (B) fast growing uniform colony on V8A.

Morphotype M3

Isolates grouped in morphotype M3 had slow growing colonies (Figure 3.5) after 14 days on PDA. These isolates had globose, ellipsoid, ovoid and obpyriform shaped sporangia and simple sporangia branching with basal attachment. Sporangia proliferation was internal, extended and nested with non-papillate sporangia.

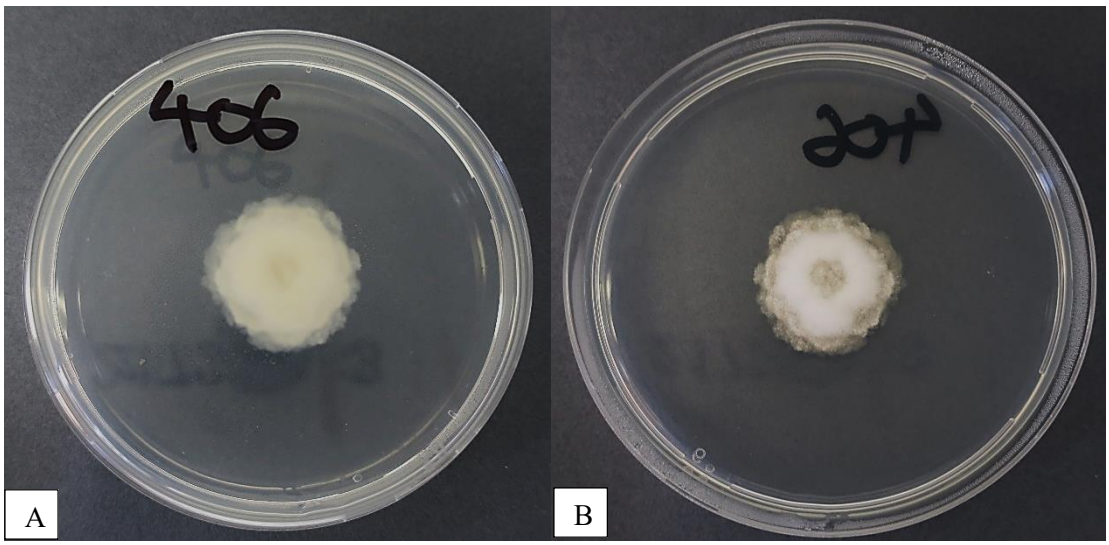

Figure 3.5: Morphotype M3, 14 day-old slow growing uniform colony on PDA; (A) bottom view and (B) top view.

This group was further divided into four subgroups i.e., M3a, M3b, M3c and M3d based on the difference in colony morphology of 7 day-old colonies on PDA and V8A. Isolates grouped into M3a (Figure 3.6) were slow growing with colonies having fine rosette patterns on PDA, with fast growing colonies with a radiate pattern on V8A. Isolates classed as M3b (Figure 3.7) had very slow growing rosette colonies on PDA with medium growing radiating colonies on V8A. M3c isolates (Figure 3.8) had very slow growing uniform colonies on PDA with medium sized uniform colonies on V8A after 7 days. Isolates grouped into M3d (Figure 3.9) had slow growing rosette colonies on PDA and medium growing uniform colonies on V8A.

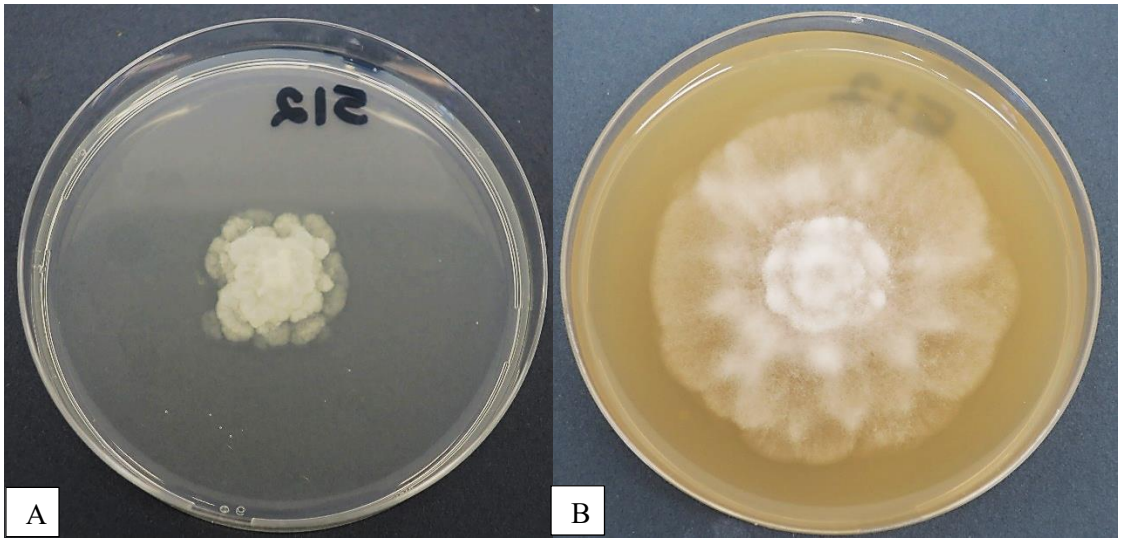

Figure 3.6: Morphotype M3a, 7 day-old colonies; (A) slow growing rosette colony on PDA, and (B) fast growing radiate colony on V8A.

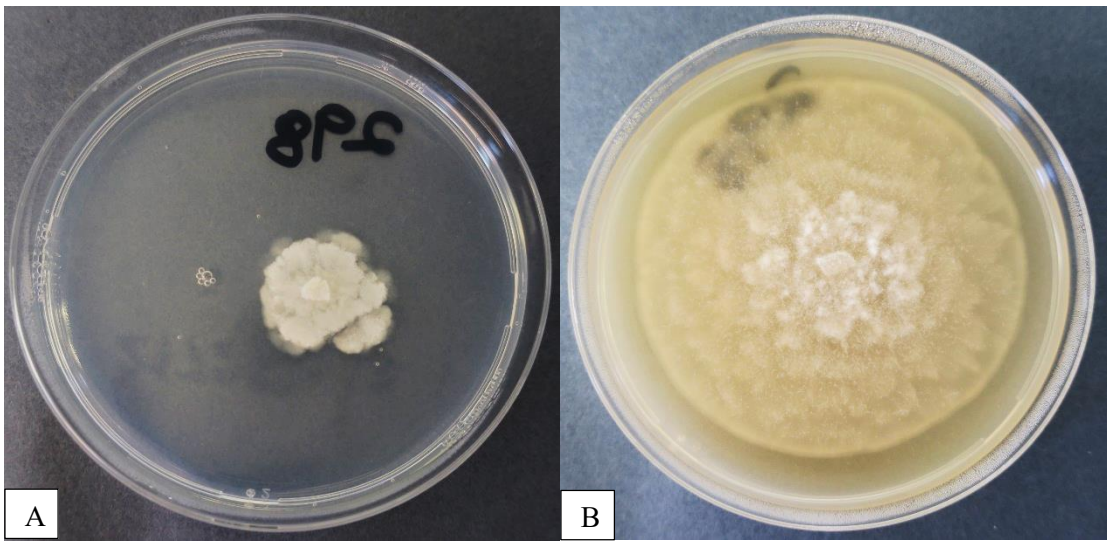

Figure 3.7: Morphotype M3b, 7 day-old colonies; (A) very slow growing rosette colony on PDA, and (B) medium growing radiating colony on V8A.

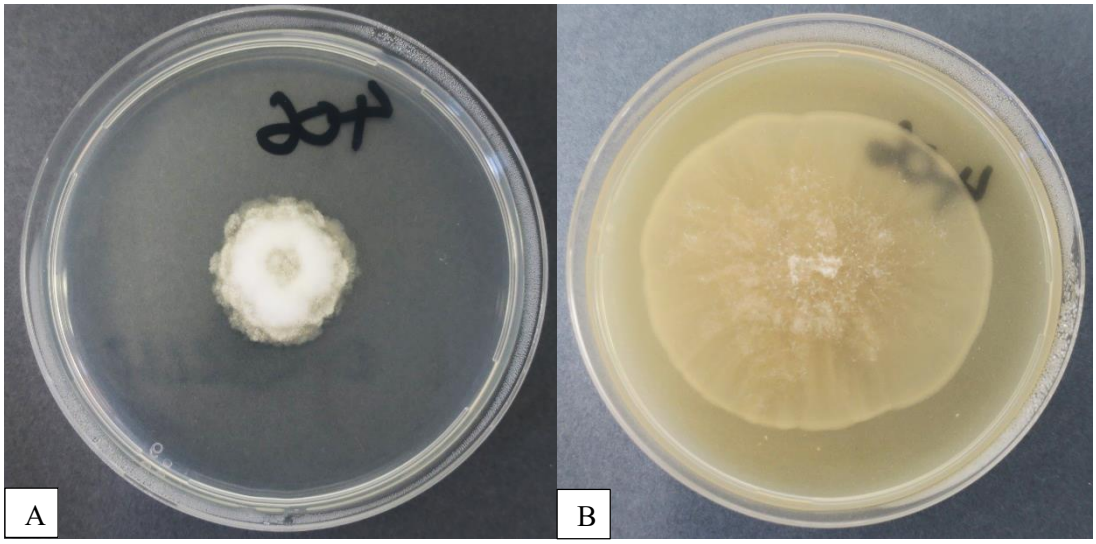

Figure 3.8: Morphotype M3c, 7 day-old colonies; (A) very slow growing uniform colony on PDA, and (B) medium growing uniform colony on V8A.

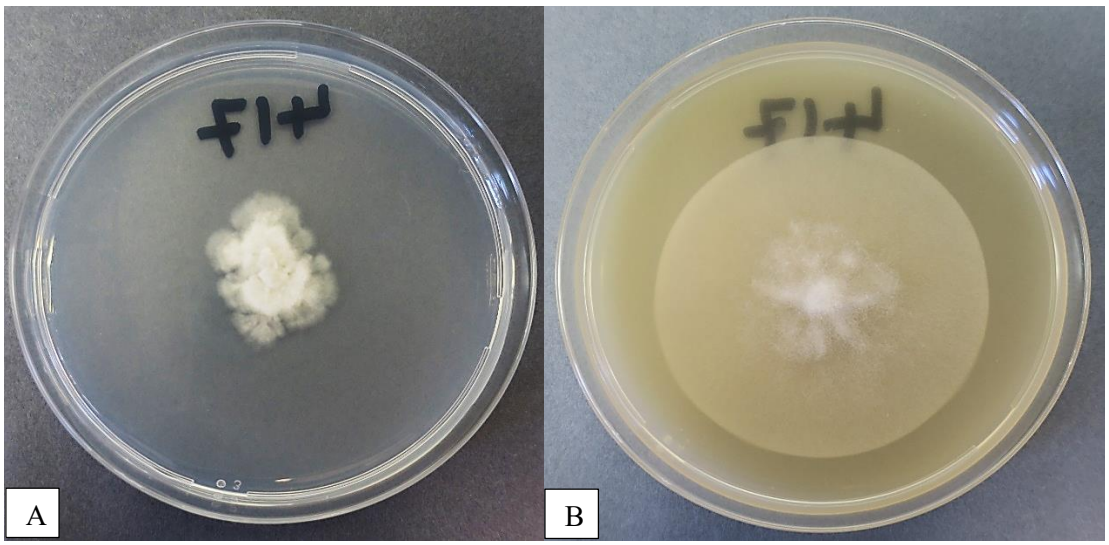

Figure 3.9: Morphotype M3d, 7 day-old colonies; (A) slow growing rosette colony on PDA, and (B) medium growing uniform colony on V8A.

Morphotype M4

Isolates in Morphotype M4 (Figure 3.10) had fast growing rosette colonies with globose, ellipsoid, ovoid and pyriform shaped sporangia. Sporangia had simple branching with basal attachment, internal, extended and nested proliferation and non-papillate sporangia.

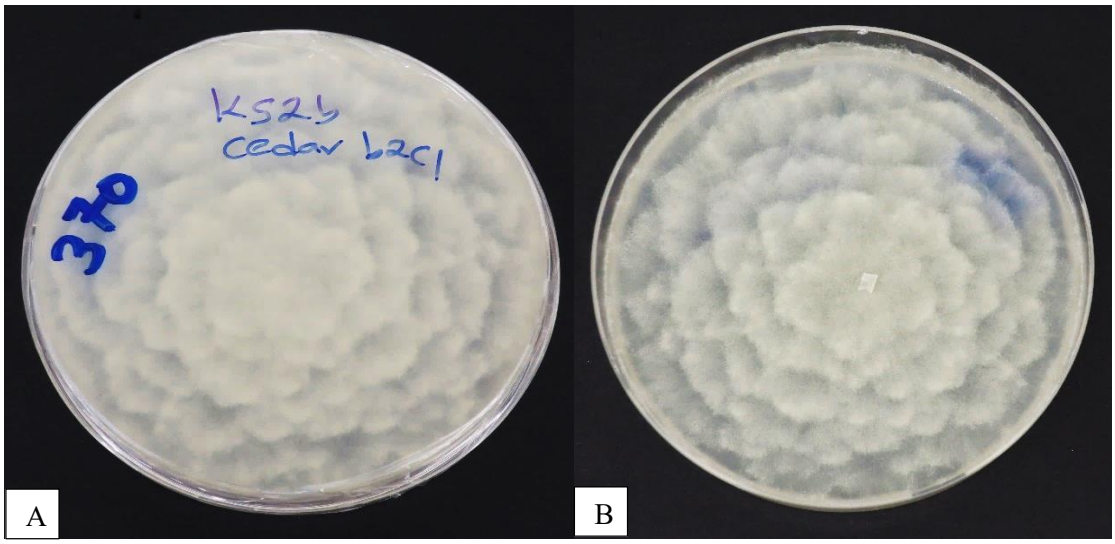

Figure 3.10: Morphotype 4, 14 day-old fast growing rosette colony on PDA; (A) bottom view and (B) top view.

Based on the morphology of 7 day-old PDA and V8A colonies, morphotype 4 was regrouped into two sub-groups i.e., M4b and M4c. M4b isolates (Figure 3.11) had medium growing rosette colonies on PDA and medium radiating colonies on V8A. Isolates grouped into morphotype M4c (Figure 3.12) had fast growing rosette colonies on PDA and fast growing rosette colonies on V8A. No isolates were identified for subgroup M4a.

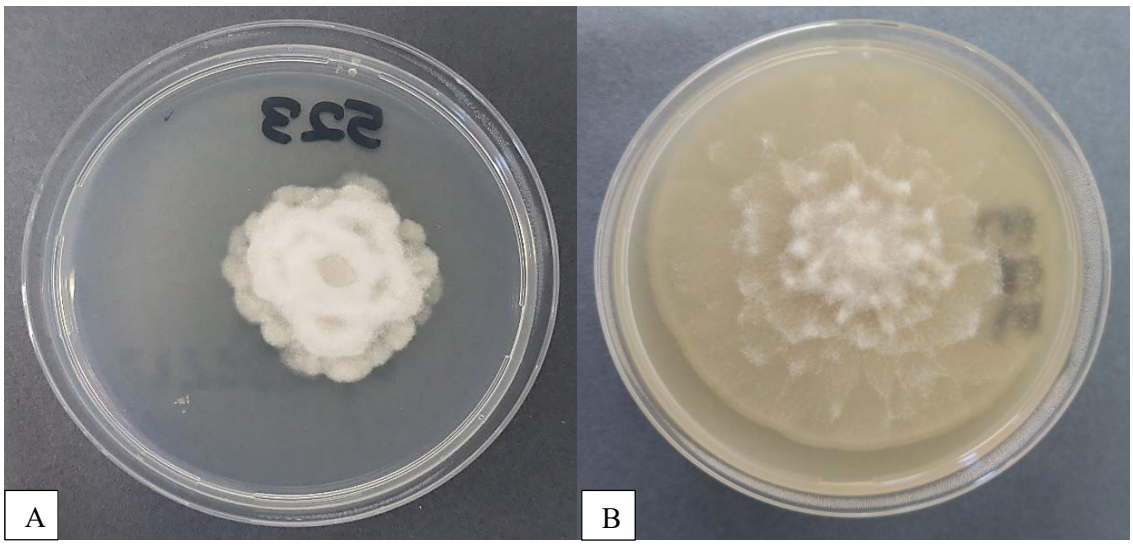

Figure 3.11: Morphotype M4b, 7 day-old colonies; (A) medium growing rosette colony on PDA, and (B) medium growing radiate colony on V8A.

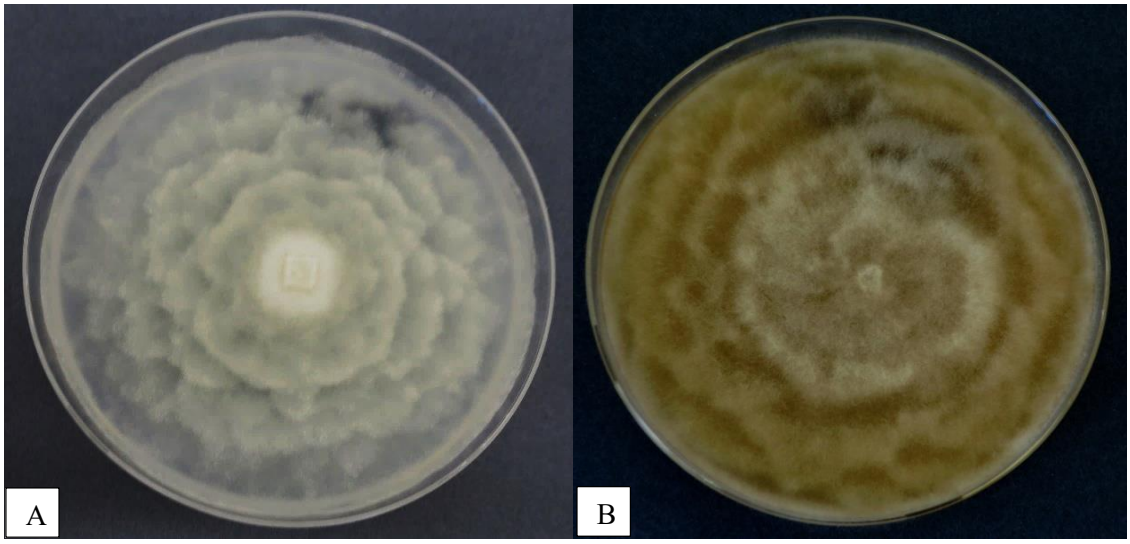

Figure 3.12: Morphotype M4c, 7 day-old colonies; (A) fast growing rosette colony on PDA, and (B) fast growing fine rosette colony on V8A.

3.3.2 *Phytophthora* sp. DNA identification

Around 10% to 20% of isolates from each morphotype group were randomly selected for DNA identification (Table 3.1; Appendix A.3.2) to confirm the *Phytophthora* species identification. The ITS results were used to confirm that the isolates from the morphotype groups were *Phytophthora* spp. and isolates from each ITS group was selected for confirmation of the *Phytophthora* spp. using the sequence of the *coxI* gene (Figure 3.13; Table 2.4; Section 2.2.6.2). A total of 147 *Phytophthora* isolates from 8 *Phytophthora* spp. or hybrids were recovered from the 25 sites sampled in the seven rivers, streams, and lake. These were *Ph. gonapodyides, Ph. bilorbang, Ph. lacustris, Ph. amnicola, Ph. chlamydospora*, *Ph. chlamydospora x Ph. amnicola* hybrid, *Ph. thermophila x Ph. amnicola* hybrid and *Ph. chlamydospora x Ph. thermophila* hybrid.

For morphotype M2, two subgroups were obtained, M2a and M2c. The 22 M2a isolates were identified as *Pythium* spp. based on the β tubulin sequence (Table 3.1). For the 93 isolates from subgroup M2c that did not produce sporangia; all isolates were tested for the presence of a band (1200 bp) on agarose gel after PCR using *Phytophthora*-specific ITS primers 18ph2F and 28ph2R (Figure 3.14; Table 2.4; Section 2.2.6.2). Eleven isolates produced bands of the expected 1200 bp size indicating they were *Phytophthora* sp. and were identified based on β-tubulin primer gene sequence TUBUF2 and TUBUR1 (Figure 3.15; Table 2.4; Section 2.2.6.2) as they were suspected to be hybrids. However, the sequencing results showed that these isolates were *Ph. lacustris* (one isolate), *Ph. gonapodyides* (four isolates), *Ph. amnicola* (three isolates), *Ph. chlamydospora* (two isolates) and *Ph. thermophila x Ph. amnicola* (one isolate). While 10 isolates of the remaining isolates that did not give a band of the expected size (1200 bp using the *Phytophthora* specific primer; Figure 3.14) were randomly selected for DNA identification using sequencing of the β-tubulin gene region, and all ten isolates were identified as *Pythium* spp. (Table 3.1). Amplification of the β-tubulin gene region using TUBUF2 and TUBUR1 for both *Pythium* and *Phytophthora* isolates produced bands of expected size, 989 bp.

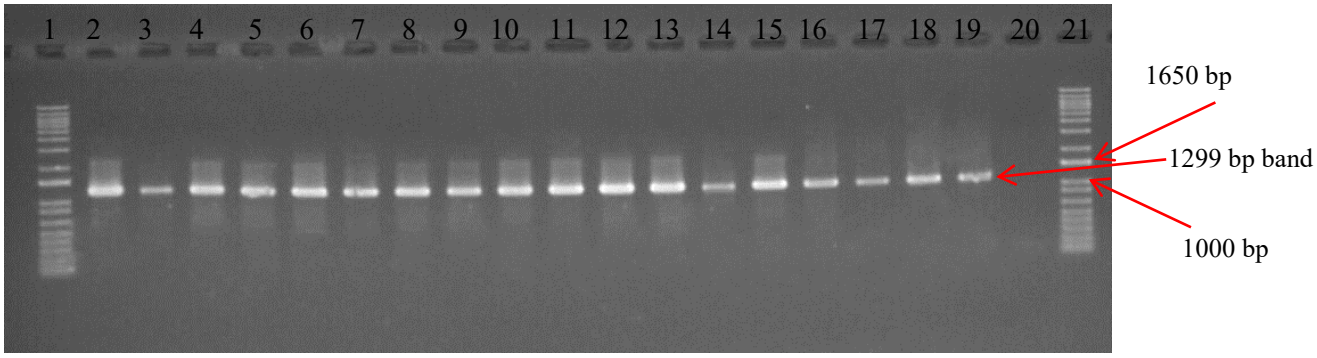

Figure 3.13: PCR products of the *coxI* gene of *Phytophthora* isolates using *coxI* gene primers (FM 77 and FM 84) separated on 1% agarose gel. *Phytophthora* isolates produced a band at 1299 bp bands. Lanes: 1 and 21: 1 Kb Plus DNA ladder, 2-11: 2-19: *Phytophthora* spp. at 1299 bp, 20: negative control (no DNA).

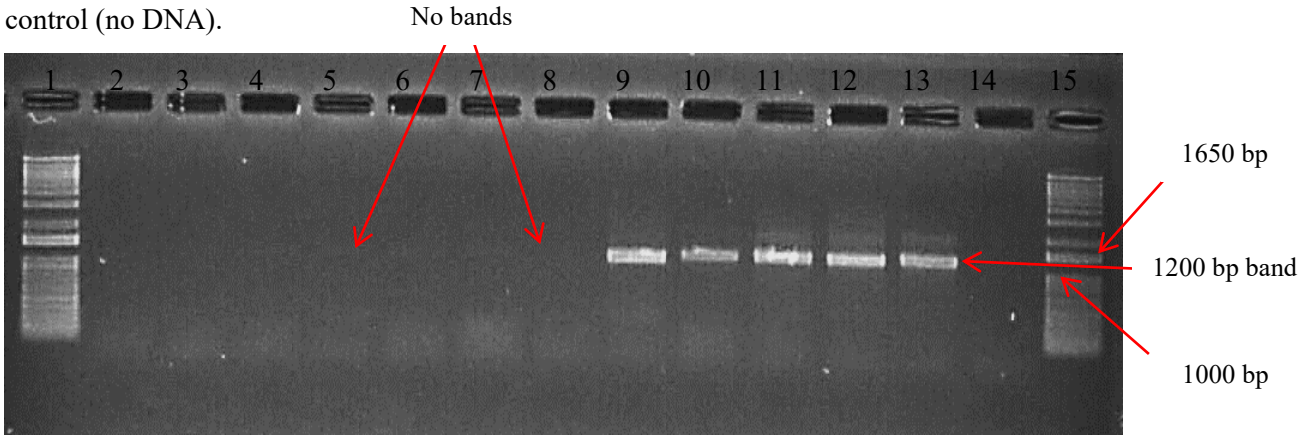

Figure 3.14: PCR products of the internal transcribed spacer (ITS) regions of isolates using *Phytophthora* specific primers (18ph2F and 28ph2R) separated on a 1% agarose gel. Amplification of *Phytophthora* spp. is indicated by a band at 1200 bp. Lanes: 1 and 15: 1 Kb Plus DNA ladder, 2-8:

Pythium spp. with no amplification; 9-13: *Phytophthora* spp. band at 1200 bp, 14: negative control (no DNA).

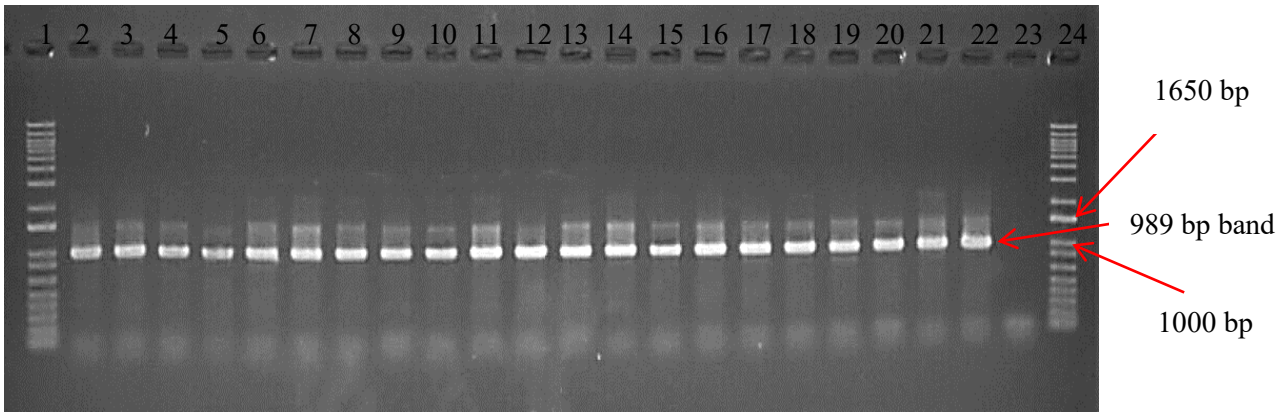

Figure 3.15: PCR products of the β-tubulin region of *Phytophthora* and *Pythium* isolates using β-tubulin primers (TUBUF2 and TUBUR1) separated on a 1% agarose gel resulting in 989 bp band. Lanes: 1 and 24: 1 Kb Plus DNA ladder, 2-11: *Pythium* spp. at 989bp; 12-22: *Phytophthora* spp. at 989bp bp, 23: negative control (no DNA).

A total of 90 isolates were identified in group M3 and the species were confirmed based on the results of *cox I* gene sequences and morphology. The 43 isolates were in subgroup M3a were identified as *Ph. thermophila x Ph. amnicola* hybrid and *Ph. gonapodyides*. Based on the data from Chapter 2 (Section 2.3.3), which showed that isolates identified based on the ITS sequence as *Ph. chlamydospora* were confirmed to be *Ph. thermophila x Ph. amnicola* hybrids based on *cox I* gene region sequences, isolates 367 and 435 which were identified as *Ph. chlamydospora* from the ITS sequence were identified as *Ph. thermophila x Ph. amnicola* hybrids. In addition, one *Ph. bilorbang* isolate was also identified in M3a.The M3b subgroup had 31 isolates identified as being either *Ph. chlamydospora* x *Ph. amnicola* hybrids or *Ph. chlamydospora* (100% sequence identity for the ITS gene region). Subgroup M3c had a total of 13 isolates identified as *Ph. thermophila* x *Ph. chlamydospora* or *Ph. amnicola x Ph. chlamydospora* hybrids. While subgroup M3d had the least number of isolates (three) which was identified as *Ph. chlamydospora* x *Ph. amnicola* hybrids. Morphotype M4 had 57 isolates with both M4b (45 isolates) and M4c (12 isolates) subgroups identified as *Ph. lacustris, confirming* the identity determined in Chapter 2 (Section 2.3.3).

Table 3.1: Identification of the isolates from the different morphological groups obtained from 25 sites sampled in Canterbury waterways based on sequencing of the ITS, *coxI* and β-tubulin gene regions.

Morpho-type	**Sub-groups**	**Isolate ID**	**ITS ID**	**Accession**	***coxI* ID**	**Accession**	**β-tubulin ID**	**Accession**
M2	M2a	461					*Py. dissotocum* (98%)	KJ595479.1
(105 isolates)	(22 isolates)	369					*Py. dissotocum* (99%)	KJ595479.1
	M2cPy*	322					*Py. dimorphum* (97%)	KJ595454.1
	(83 isolates)	424					*Py. dissotocum* (99%)	KJ595479.1
		414					*Py. dissotocum* (99%)	KJ595479.1
		278					*Py. dissotocum* (99%)	KJ595479.1
		273					*Py. dissotocum* (99%)	KJ595479.1
		458					*Py. dimorphum* (97%)	KJ595454.1
		460					*Py. dissotocum* (97%)	DQ071306.1
		385					*Py. dissotocum* (98%)	KJ595479.1
		386					*Py. dissotocum* (98%)	KJ595479.1
		462					*Py. dissotocum* (98%)	KJ595479.1
	M2cPhy*(11 isolates)	289					*Ph. amnicola* (100%)	JQ029951.1
		259					*Ph. amnicola* (100%)	JQ029951.1
		392					*Ph. amnicola* (100%)	JQ029951.1
		325					*Ph. gonapodyides* (99%)	EU080119.1
		416			*Ph. gonapodyides* (100%)	JN547642.1	*Ph. gonapodyides* (99%)	JN547581.1
		382			*Ph. thermophila* x *Ph. amnicola* strain MUCC780 (100%)	JQ936803.1	*Ph. lacustris* (99%)	JN547618.1

Table 3.1 continued

Morpho-type	Sub-groups	Isolate ID	ITS ID	Accession	*coxI* ID	Accession	Beta Tub ID	Accession
	M2cPhy *(11 isolates)	279			*Ph. thermophila* x *Ph. amnicola* strain MUCC781 (99%)	JQ936804.1	*Ph. thermophila* x *Ph. amnicola* strain DH150 (98%)	KM883102.1
		309					*Ph. gonapodyides* (99%)	JN547581.1
		483					*Ph. gonapodyides* (99%)	JN547581.1
		328	*Phytophthora* taxon PgChlamydo * (99%)	HM004224.1	*Phytophthora* taxon PgChlamydo *(98%)	HQ012879.1	*Phytophthora* taxon Pgchlamydo (99%)	JN547617.1
		324	*Phytophthora* taxon PgChlamydo * (99%)	HM004224.1			*Phytophthora* taxon Pgchlamydo* (99%)	JN547617.1
M3	M3a	281	*Ph. gonapodyides* (98%)	HM004231.1				
(90 isolates)	(43 isolates)	395	*Phytophthora* taxon Oaksoil (99%)	HM004234.1	*Ph. bilorbang* (99%)	JN547644.1		
		463	*Ph. gonapodyides* (98%)	HM004231.1	*Ph. gonapodyides* (100%)	JN547642.1		
		300	*Ph. gonapodyides* (98%)	HM004231.1				
		367	*Phytophthora* taxon PgChlamydo (99%)	HM004224.1				
		288	*Phytophthora gonapodyides* (98%)	HM004231.1				
		435	*Phytophthora* taxon PgChlamydo (98%)	HM004224.1				

Table 3.1 continued

Morpho-type	**Sub-groups**	**Isolate ID**	**ITS ID**	**Accession**	***coxI* ID**	**Accession**	**Beta Tub ID**	**Accession**
M3	M3a	292	*Ph. gonapodyides* (98%)	HM004231.1	*Ph. gonapodyides* (100%)	MG721475.1		
(90 isolates)	(43 isolates)	325					*Ph. gonapodyides* (99%)	EU080119.1
		271	*Ph. gonapodyides* (98%)	HM004231.1				
		416			*Ph. gonapodyides* (100%)	JN547642.1	*Ph. gonapodyides* (99%)	JN547581.1
		382			*Ph. thermophila* x *Ph. amnicola* strain MUCC780 (100%)	JQ936803.1	*Ph. lacustris* (99%)	JN547618.1
		279			*Ph. thermophila* x *Ph. amnicola* strain MUCC781 (99%)	JQ936804.1	*Ph. thermophila* x *Ph. amnicola* strain DH150 (98%)	KM883102.1
		309					*Ph. gonapodyides* (99%)	JN547581.1
		483					*Ph. gonapodyides* (99%)	JN547581.1
M3	M3b (31 isolates)	291	*Phytophthora* taxon PgChlamydo (97%)	HM004224.1	*Phytophthora* taxon Pgchlamydo x *Ph. amnicola* strain MUCC778 (100%)	JQ936799.1		
		295	*Phytophthora* taxon PgChlamydo (100%)	KJ755194.1				

Table 3.1 continued

Morpho-type	Sub-groups	Isolate ID	ITS ID	Accession	*coxI* ID	Accession	Beta Tub ID	Accession
M3	M3b (31 isolates)	328	*Phytophthora* taxon PgChlamydo * (99%)	HM004224.1	*Phytophthora* taxon PgChlamydo *(98%)	HQ012879.1	*Phytophthora* taxon Pgchlamydo (99%)	JN547617.1
		372	*Phytophthora* taxon PgChlamydo * (99%)	HM004224.1				
		412	*Phytophthora* taxon PgChlamydo * (99%)	HM004224.1				
		400	*Phytophthora* taxon PgChlamydo* (99%)	HM004224.1				
		336	*Phytophthora* taxon PgChlamydo * (99%)	HM004224.1				
		324	*Phytophthora* taxon PgChlamydo * (99%)	HM004224.1			*Phytophthora* taxon Pgchlamydo* (99%)	JN547617.1
		262	*Phytophthora* taxon PgChlamydo * (99%)	HM004224.1				
M3	M3c	427	*Ph. gonapodyides* (98%)	HM004231.1				
	(13 isolates)	267	*Ph. gonapodyides* (98%)	HM004231.1				
		334	*Ph. gonapodyides* (98%)	HM004231.1				
		361	*Ph. gonapodyides* (96%)	HM004231.1	*Ph. thermophila* x *Phytophthora* taxon Pgchlamydo* strain MUCC783 (100%)	JQ936801.1		

Table 3.1 continued

Morpho-type	Sub-groups	Isolate ID	ITS ID	Accession	*coxI* ID	Accession	Beta Tub ID	Accession
M3	M3c	352	*Phytophthora* taxon PgChlamydo* (99%)	HM004224.1	*Ph. amnicola* x *Phytophthora* taxon PgChlamydo* strain MUCC774 (99%)	JQ936797.1		
M3	M3d (3 isolates)	280	*Ph. gonapodyides* (98%)	HM004231.1	*Ph. amnicola* x *Phytophthora* taxon PgChlamydo* strain MUCC774 (100%)	JQ936797.1		
		296	*Ph. gonapodyides* (98%)	HM004231.1				
M4 (57 isolates)	M4b (45 isolates)	374	*Ph. lacustris* (99%)	HM004219.1				
		310	*Ph. lacustris* (99%)	HM004219.1				
M4	M4c (12 isolates)	316			*Ph. lacustris* (100%)	JQ626633.1		

**Phytophthora* taxon PgChlamydo has been redesignated as *Ph. chlamydospora* (Hansen *et al.*, 2015).

* M2cPy isolates identified as *Pythium* species which did not produce a band using *Phytophthora* specific primers, and M2cPhyt isolates identified as *Phytophthora* species which produced a 1200 bp band using *Phytophthora* specific primers

3.3.3 Diversity of *Phytophthora* spp. in the waterways

Phytophthora spp. were recovered from all the seven waterways and from 22 of the 25 sites (Figure 3.16) baited for *Phytophthora*. No *Phytophthora* spp. isolates were recovered from either Ashburton River site 4 or Selwyn River sites 7 and 8. *Phytophthora lacustris* (56 isolates) was the most commonly isolated species recovered from 19 of the 25 sites. This was followed by *Ph. gonapodyides* (36 isolates) recovered from 14 sites, *Ph. chlamydospora* x *Ph. amnicola* hybrid (33 isolates) recovered from 15 sites and *Ph. thermophila x amnicola* hybrid (12 isolates) recovered from eight sites (Figure 3.17).

Low numbers of *Ph. chlamydospora* x *Ph. thermophila* hybrid (three isolates; from three sites), *Ph. chlamydospora* (three isolates; from three sites), *Ph. amnicola* (three isolates; three sites) and *Ph. bilorbang* (one isolate) was also recovered. All these species were recovered from different sites that had varying land use type and vegetation. The three species, *Ph. chlamydospora* x *Ph. thermophila* hybrid, *Ph. chlamydospora* and *Ph. amnicola*, were recovered from Kaituna Valley site 3 and other areas that had trees i.e., Selwyn River site 1 and 4, Prices Valley site 4, Halswell River site 1, Kaituna Valley site 2. While *Ph. bilorbang* was only recovered from Selwyn River site 1 which is a recreational reserve (Figure 3.15).

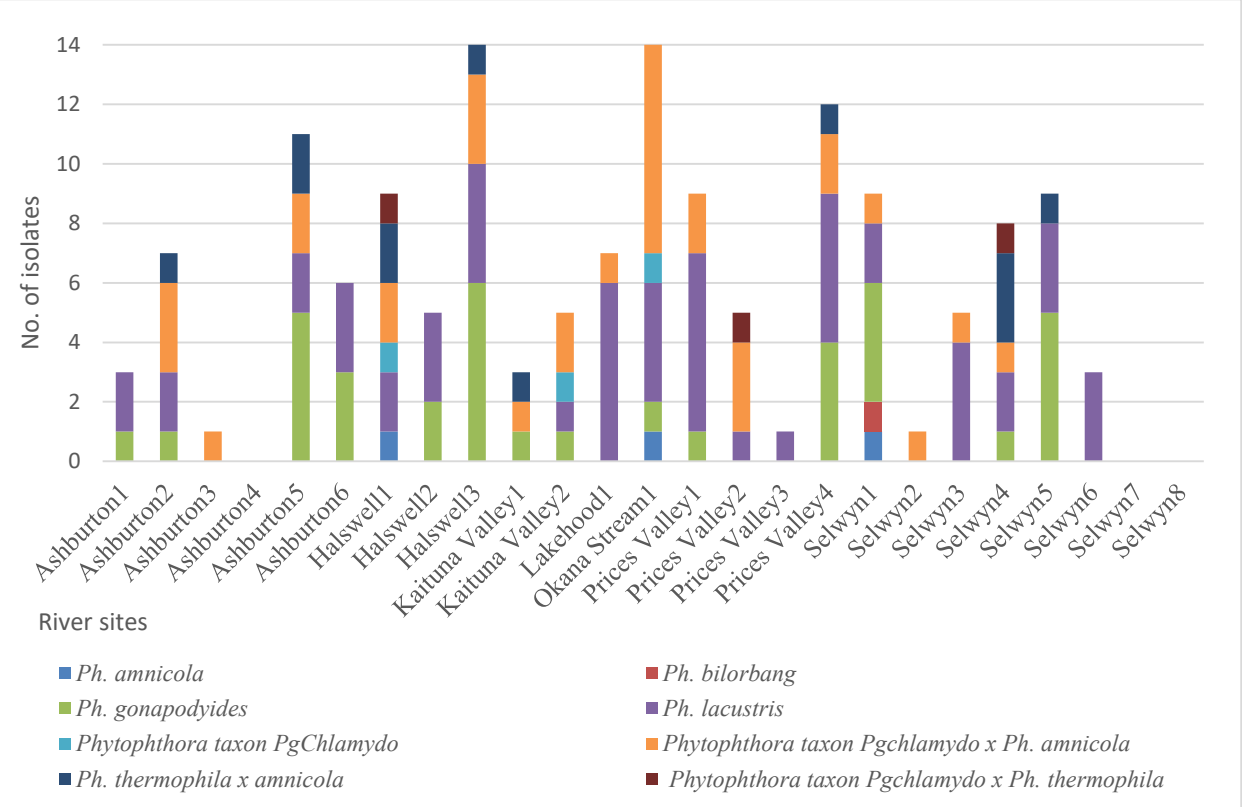

Figure 3.16: Relative frequency of the different *Phytophthora* spp. or hybrids isolated from 25 sites sampled in the 6 waterways using the laboratory baiting method.

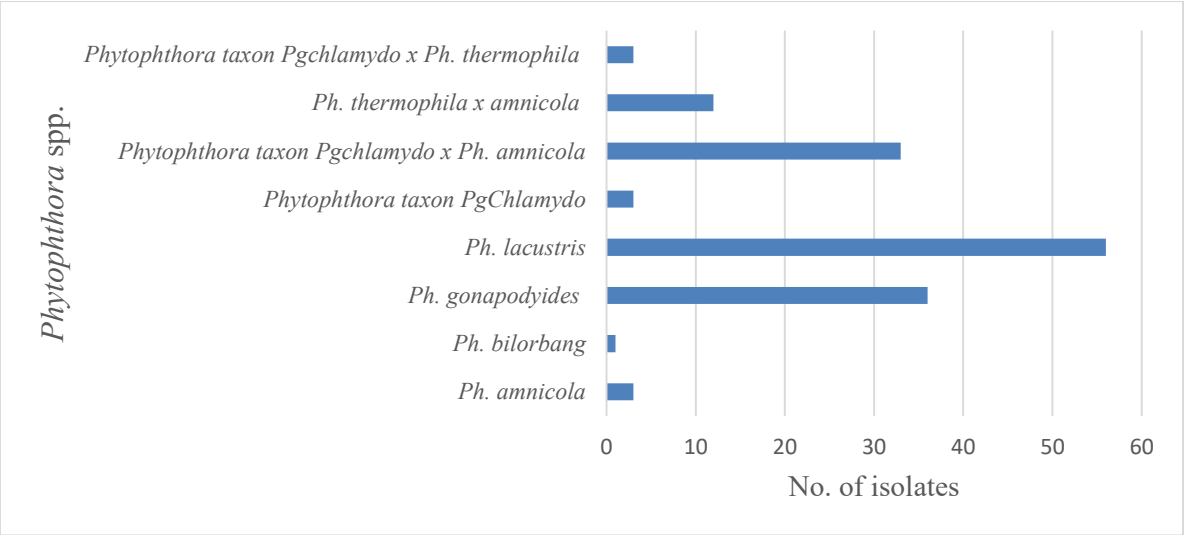

Figure 3.17: Frequency of the eight *Phytophthora* spp.or hybrids isolated using the laboratory baiting method from water samples from the six waterways.

The number of *Phytophthora* species recovered was significantly affected by bait type (P <0.001) and site (P < 0.001), however, there was no significant interaction between bait type and site (P= 0.36; Appendix 3.3; Table A1) on number of *Phytophthora* spp. recovered. The bait type and site interaction in this analysis was due to inclusion of sites and bait type data that did not recover any *Phytophthora* in analysis.

When the three sites that did not recover any *Phytophthora* (Ashburton site 4, Selwyn site 7 and 8) were removed from the analysis, a significant difference (P = 0.0036; Appendix A.3.3; Table A2) was observed in the number of *Phytophthora* spp. (diversity) isolated from the 22 river sites. Multiple comparisons of mean (at 90% confidence) revealed that Kaituna Valley site 3 differed from Ashburton site 3 (P = 0.06), Prices Valley site 3 (P =0.06), and Selwyn site 2 (P = 0.06; Appendix A.3.3; Table A3).

All the sites and bait type that did not recover any *Phytophthora* spp. were then removed from the analysis and this showed that only bait type (P=0.004) had an effect on the number of *Phytophthora* spp. recovered irrespective of the site and there was no significant interaction between bait type and site (P = 0.8) on the number of species recovered (Appendix A.3.3; Table A4).

Analysis was also done to see if there was any difference in the number of *Phytophthora* isolates recovered from the different river sites. Data analysis (without sites and bait types that did not recovered any *Phytophthora*) showed that there was an effect of bait type (P=0.01) on the number of *Phytophthora* isolates recovered. There was no significant effect of site (P=0.50) and no significant interaction between bait type and site (P=0.94; Appendix A.3.3; Table A5) on the number of *Phytophthora* isolates recovered.

3.3.3.1 Selwyn River (Waikirikiri)

3.3.3.1.1 *Phytophthora* isolated from Selwyn River

The Selwyn River was one of the major rivers sampled, with eight sites sampled. The river flows from the Canterbury foothills above Whitecliffs snaking its way across the farmlands in Canterbury Plains before entering Lake Ellesmere. Water sampling therefore was done from the foothills and down towards Lake Ellesmere (Figure 3.18). A total of 35 *Phytophthora* isolates representing seven *Phytophthora* spp. (including three *Phytophthora* hybrids) were obtained from six of the eight sites. Site 5 was up in the foothills and nine *Phytophthora* isolates representing three *Phytophthora* spp., *Ph. lacustris* (three isolates), *Ph. gonapodyides* (one isolate) and *Ph. thermophila x amnicola* hybrid (one isolate) were recovered. Site 6 (2 km from site 5) was also in the foothills and three *Phytophthora* isolates representing one *Phytophthora* spp., *Ph. lacustris* was recovered. The next site was site 7 (15 km from site 6) and site 8 (20 km from site 7) located on the Canterbury Plains, however no *Phytophthora* isolates were recovered from these two sites. In site 2 (6.9 km away from site 8) only one *Phytophthora* isolate was recovered i.e., *Ph. chlamydospora* x *Ph. amnicola* hybrid. Five *Phytophthora* isolate representing two *Phytophthora* spp., *Ph. lacustris* (four isolates) and *Ph. chlamydospora* x *Ph. amnicola* hybrid (one isolate), was recovered from site 3 (5 km away from site 2). Nine *Phytophthora* isolates were recovered from site 1 (Figure 3.19) representing five *Phytophthora* spp., *Ph. lacustris* (two isolates), *Ph. gonapodyides* (four isolates), *Ph. amnicola* (one isolate), *Ph. bilorbang* (one isolate) and *Ph. chlamydospora* x *Ph. amnicola* hybrid (one isolate). Site 4 was the last site sampled for the Selwyn River located 3 km away from site 1 and 3 km away from Lake Ellesmere. A total of eight *Phytophthora* isolates representing five *Phytophthora* spp., *Ph. lacustris* (two isolates), *Ph. gonapodyides* (one isolate), *Ph. chlamydospora* x *Ph. amnicola* hybrid (one isolate), *Ph. chlamydospora* x *Ph. thermophila* hybrid (one isolate), and *Ph. thermophila x amnicola* hybrid (three isolate).

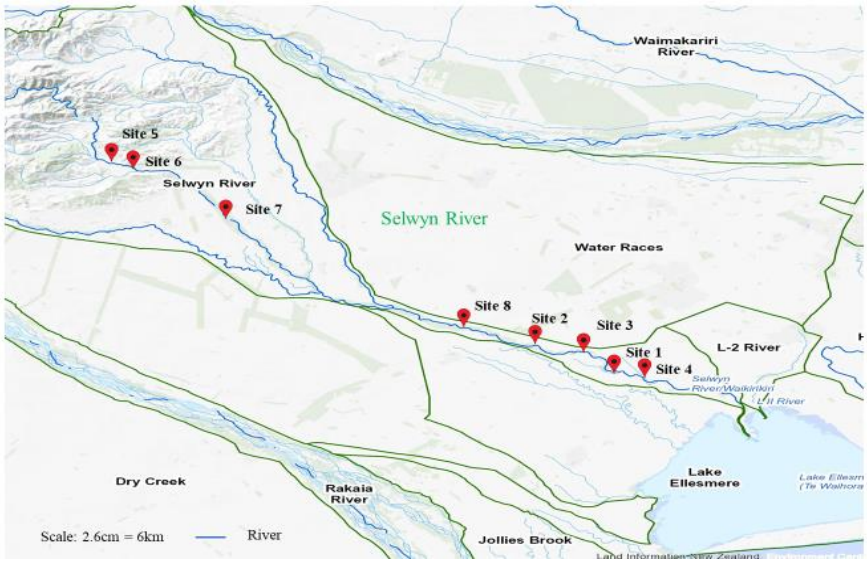

Figure 3.18: Water sampling of eight sites in the Selwyn River, Source: Land Information New Zealand (2019).

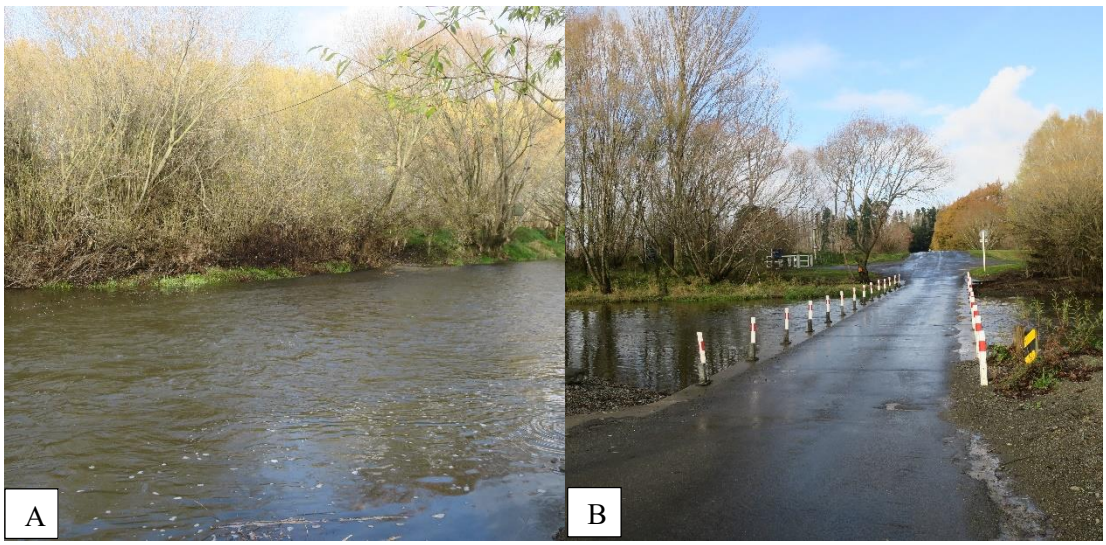

Figure 3.19: Water sampling done at Coes Ford Reserve (Selwyn River site 1), (A) water sampling area, and (B) surrounding vegetation.

3.3.3.1.2 Description of land use type for the Selwyn River water sampling sites

Five out the eight sites (sites 2, 5,6,7,8) sampled in the Selwyn River were from farming areas (Table 3.2) and mostly had sheep, dairy, beef and pasture with some sites having varying vegetation from pine forests, beech forests/native bush to willows and other bushes. Two of the sites sampled were camping sites/reserves (site 1 and site 3) containing some native and exotic trees/bush, however, these areas were also surrounded by sheep, dairy, beef, cropping, pasture (site 1), cropping, trees and bush (site 3). While site 4 was around a residential area (Selwyn Huts) near Lake Ellesmere surrounded with willow trees and bushes.

Table 3.2: Land use types for the eight sites sampled along the Selwyn River/Waikirikiri

River sites	Area description	Land use
Site 1	Coes Ford Reserve	Camping site with trees and bushes surrounded by dairy, sheep, beef, cropping and pasture
Site 2	Farming area	Surrounded by sheep, dairy, brassica, beef, beech and pine trees
Site 3	Chamberlins Ford Reserve	Camping site, surrounded by trees and bushes
Site 4	Selwyn Huts (residential area)	Near Lake Ellesmere, surrounded by willow trees and bushes
Site 5	Farming area (Whitecliffs hill)	Passes through sheep, beef, pine and beech forest
Site 6	Farming area (Whitecliffs hill)	Passes through sheep, beef, pine, willow and native bush
Site 7	Farming area	Passes through dairy, sheep and surrounded by bushes, pine and beech forest
Site 8	Farming area	Passes dairy, pasture and surrounded by mostly pine and bush

3.3.3.2 Ashburton River (Hakatere)

3.3.3.2.1 *Phytophthora* isolated from Ashburton River

The second major river surveyed was the Ashburton river, where water sampling was done from the Canterbury foothills near Mount Somers and the river was followed down out towards the sea (Figure 3.20). A total of 28 *Phytophthora* isolates representing four *Phytophthora* spp. (including two *Phytophthora* hybrids) were obtained from the six sites (Figure 3.15). No *Phytophthora* isolates were recovered from Site 4 which is a Department of Conservation area (DOC) area with native bush and uphill of Mount Somers. Only one isolate identified as a *Ph. chlamydospora* x *Ph. amnicola* hybrid was recovered from site 3 (Figure 3.21A), which was further downhill from site 4 (0.6 km) and was surrounded by manuka, hebe and other native bushes and some pine trees. Site 2 (22.0 km from site 3) sampling was on flatland near Mount Somers, with seven *Phytophthora* isolates recovered representing four *Phytophthora* spp., *Ph. gonapodyides* (one isolate), *Ph. lacustris* (two isolates), *Ph. chlamydospora* x *Ph. amnicola* hybrid (three isolates) and *Ph. thermophila* x *Ph. amnicola* hybrid (one isolate). Ashburton River sites 1 (Figure 3.21 B) and 5 were on a separate river branch (North branch) from sites 2, 4 and 5 (South branch) that was flowing down from Mount Somers and meeting up in the Canterbury plains. The distance between the two river branches (site 2 on the South branch and site 1 on the North branch) was 5.1 km. From site 1 *Ph. gonapodyides* (one isolate) and *Ph. lacustris* (two isolates) were recovered, whilst from site 5 the highest number of isolates (11 isolates) were recovered representing with four *Phytophthora* spp., *Ph. gonapodyides* (five isolates), *Ph. lacustris* (two isolates), *Ph. chlamydospora* x *Ph. amnicola* hybrid (two isolates) and *Ph. thermophila* x *Ph. amnicola* hybrid (two isolates). Site 6 was sampled after the two rivers branches merged (22.1 km from site 5 and 35.1 km from site 2) and *Ph. gonapodyides* (three isolate) and *Ph. lacustris* (three isolates) were recovered.

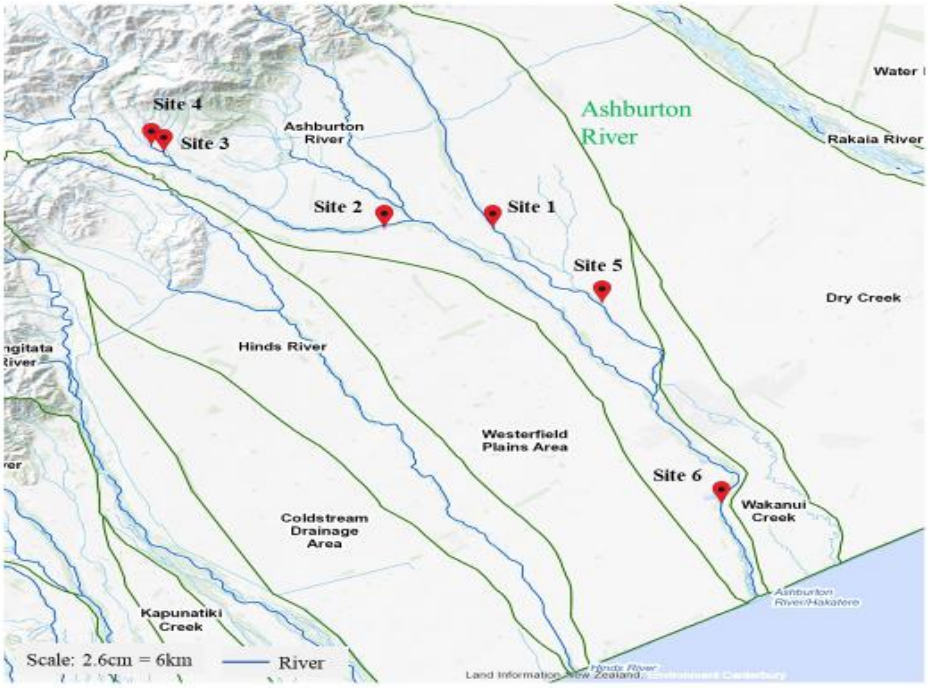

Figure 3.20: Water sampling of six sites in the Ashburton River, Source: Land Information New Zealand (2019).

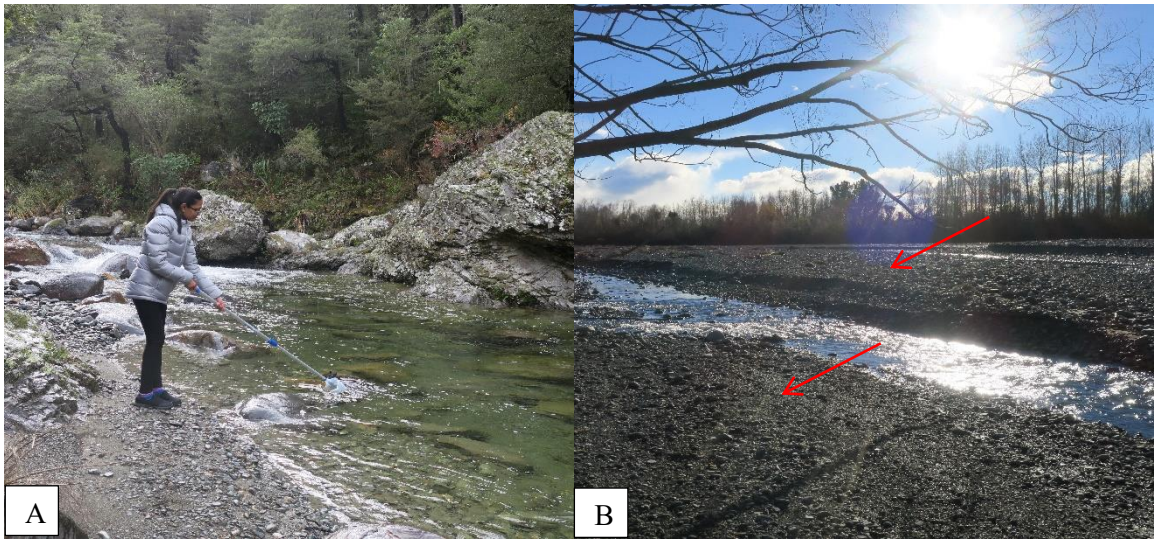

Figure 3.21: Water sampling done in Ashburton River sites (A) Woolshed creek (Ashburton site 3) and, (B) braided river with stony river banks (Ashburton site 1) marked with red arrows.

3.3.3.2.2 Description of land use type for the Ashburton River water sampling sites

Land use type surrounding the Ashburton River was similar to Selwyn River. The major farming activities included sheep, dairy, pasture, brassica, with pine, manuka, native bushes also present (Table 3.3)

Table 3.3: Land use types for the eight sites sampled along the Ashburton River/Hakatere.

River sites	Area description	Land use
Site 1	Ashburton north branch bridge	Surrounded by dairy, grazing, forest and sheep
Site 2	Ashburton south branch bridge	Surrounded by pine, bush, weeds and passes through dairy
Site 3	Woolshed Creek Conservation area Picnic area/ car park	DOC reserve area surrounded by pine, beech, manuka, hebes and other native bush
Site 4	Woolshed conservation reserve	DOC reserve area surrounded by beech, manuka, hebe and other native bush
Site 5	Farming area	Dairy, brassica, poultry, sheep and pasture
Site 6	Farming area	Cropping area, ploughed bare land, brassica, silage, blackcurrant and sheep

3.3.3.3 Banks Peninsula

Four sites in Prices Valley and three sites in Kaituna Valley were selected for water sampling in the Banks Peninsula area.

3.3.3.3.1 *Phytophthora* isolated from Prices Valley River

Of the four sites sampled, three sites (site 1, site 2 (0.4 km away from site 1) and site 3 (1.0km away from site 2)) were all located in the foothills of Prices Valley. Site 4 (3.6 km away from site 3 and 1.7 km away from Lake Ellesmere) was on the valley floor before the river emptied into Lake Ellesmere (Figure 3.22). From site 1, nine *Phytophthora* isolates were recovered representing three *Phytophthora*s spp., *Ph. lacustris* (six isolates), *Ph. gonapodyides* (one isolate) and *Ph. chlamydospora* x *Ph. amnicola* hybrid (two isolates). Five *Phytophthora* isolates were recovered from site 2, representing three *Phytophthora*s spp., being *Ph. lacustris* (one isolate), *Ph. chlamydospora* x *Ph. amnicola* hybrid (three isolates) and *Ph. chlamydospora* x *Ph. thermophila* hybrid (one isolate). Only one isolate of *Ph. lacustris* was isolated from site 3, while site 4 isolated the highest number of isolates (12 isolates) and *Phytophthora* spp. (four species.) including, *Ph. lacustris* (five isolates), *Ph. gonapodyides* (four isolates), *Ph. chlamydospora* x *Ph. amnicola* hybrid (two isolates) and *Ph. chlamydospora* x *Ph. thermophila* hybrid (one isolate).

3.3.3.3.2 Description of land use type in Prices Valley River water sampling sites

Prices Valley had three sites that were close to native bush being site 1, site 2 (Figure 3.23 A) and site 3 (Figure 3.23 B). Additionally, site 4 was also surrounded by trees such as pine and *Cupressus macrocarpa* (Monterey cypress) and located in a farming area of sheep, beef and pasture (Table 3.4).

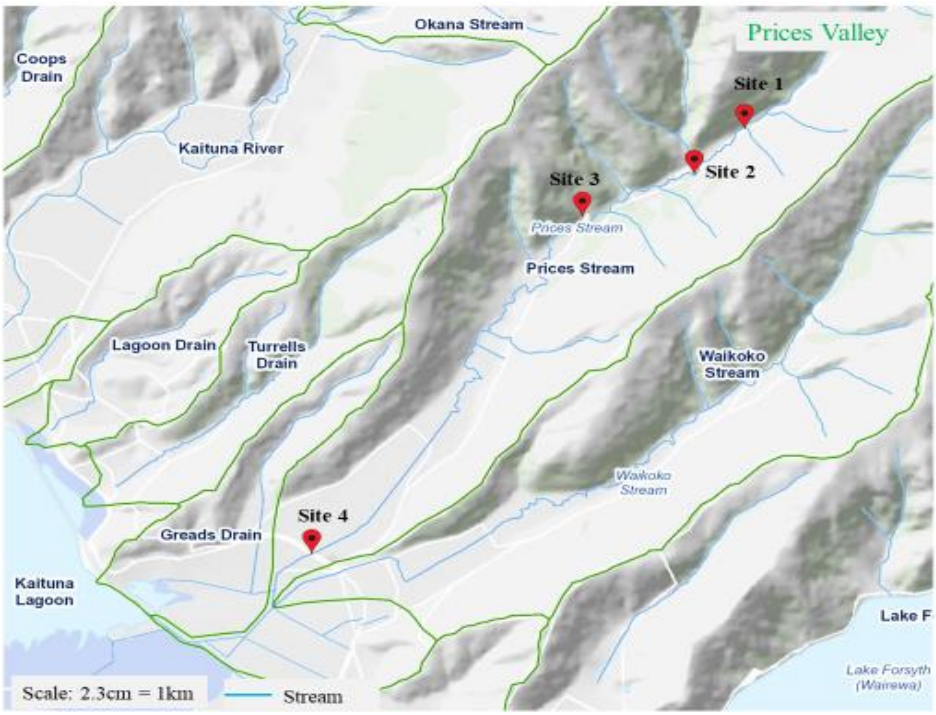

Figure 3.22: Water sampling of four sites in Prices Valley, Source: Land Information New Zealand (2019).

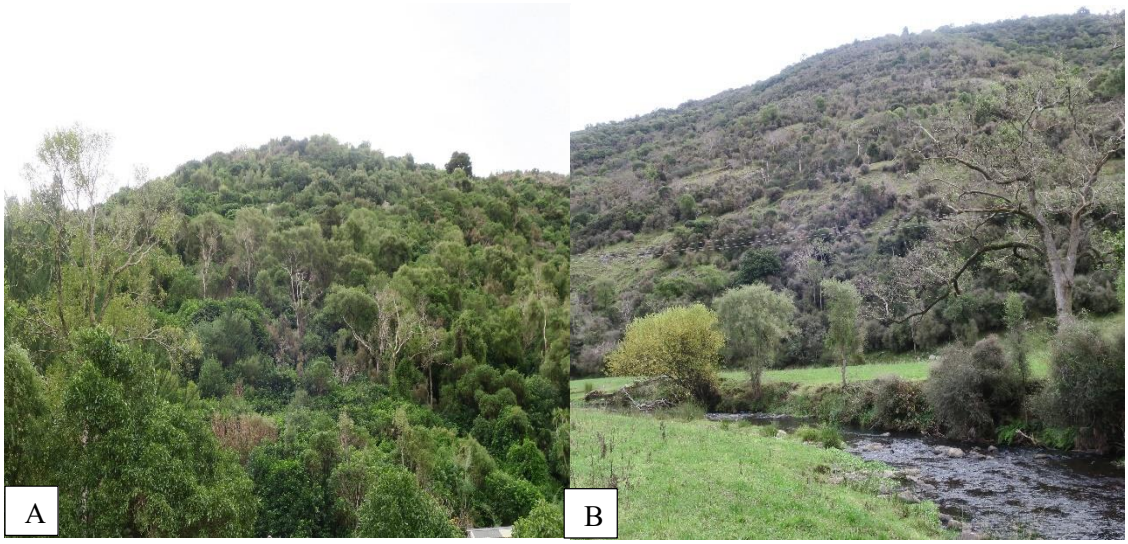

Figure 3.23: Prices Valley, (A) Regenerated young native bush near site 1 and 2, and (B) site 3 which passes through the old protected native bush.

Table 3.4: Land use types for the four sites sampled along the Prices Valley Stream.

River sites	Area description	Land use
Site 1	Regenerated young native bush	Passes through native bush and sheep grazing
Site 2	Regenerated young native bush	Passes through native bush and sheep grazing
Site 3	Old protected native bush	Passes through old native bush
Site 4	Farming area	Passes through Prices Valley, bush, sheep and beef, pine trees and cypress trees

3.3.3.4 *Phytophthora* isolated from Kaituna Valley River

Three sites were sampled in Kaituna Valley. Kaituna River site 2 was up in the foothills, with site 3, (0.9 km away from site 2) being a small stream (Okana stream) which feeds in to the Kaituna River. While Kaituna River site 1 (3.8 km away from Kaituna Valley site 3 and 0.8 km away from Lake Ellesmere) was located on the valley floor just before the river emptied into Lake Ellesmere.

From Kaituna River site 2 five *Phytophthora* isolates were recovered, representing four *Phytophthora* spp., being *Ph. lacustris* (one isolate), *Ph. gonapodyides* (one isolate), *Ph. chlamydospora* (one isolate) and *Ph. chlamydospora* x *Ph. amnicola* hybrid (two isolates). Kaituna Valley site 3 isolated the highest number of *Phytophthora* isolates (14 isolates) and *Phytophthora* spp. (five species) i.e., *Ph. lacustris* (four isolates), *Ph. gonapodyides* (one isolate), *Ph. amnicola* (one isolate), *Ph. chlamydospora* (one isolate) and *Ph. chlamydospora* x *Ph. amnicola* hybrid (seven isolates). Kaituna site 1 isolated the lowest *Phytophthora* isolates (three isolates) and *Phytophthora*s spp. (three species.) i.e., *Ph. gonapodyides* (one isolate), *Ph. chlamydospora* x *Ph. amnicola* hybrid (one isolate) and *Ph. thermophila x amnicola* hybrid (one isolate).

3.3.3.4.1 Description of Land use type in Kaituna Valley River water sampling sites

Three sites were sampled in Kaituna Valley (Figure 3.24). Sites1 and 2 sampled were surrounded by sheep, dairy and pasture (Table 3.5). Site 1 was mostly surrounded by pine (Figure 3.25A), while site 2 had regenerated native bush (Figure 3.25B). Site 3 in the Kaituna Valley was surrounded by native bush and close to an abandoned vineyard and had flowed through sheep and beef pasture.

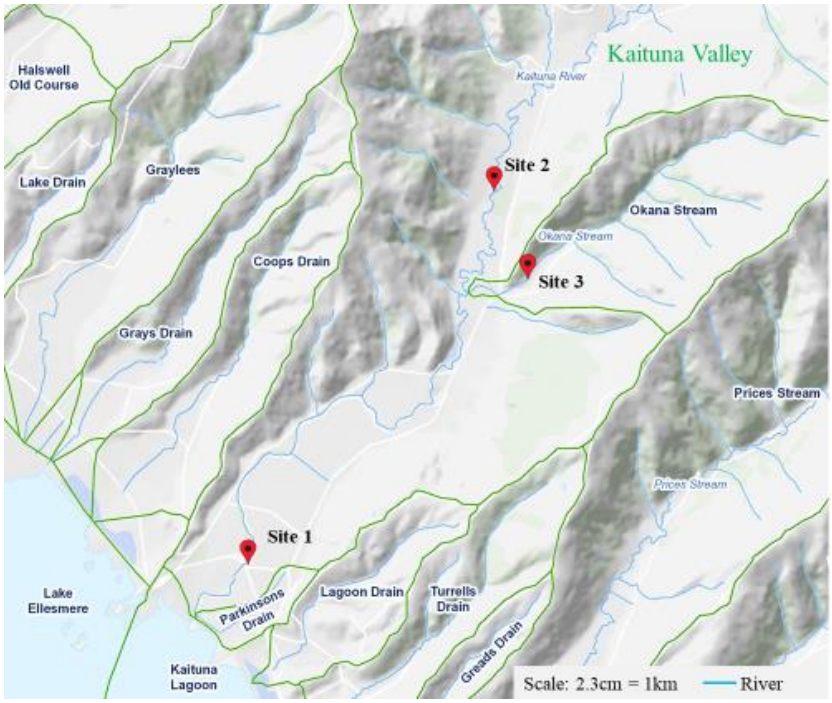

Figure 3.24: Water sampling of three sites in Kaituna Valley, Source: Land Information New Zealand (2019).

Table 3.5: Land use types for the three sites sampled along the Kaituna Valley River.

River sites	Area Description	Land use
Site 1	Farming area	Passes through dairy (Willesden Farm) but no stock on farm, sheep and pine
Site 2	Kaituna Valley science reserve	Regenerated native bush, surrounded by dairy, sheep and pasture
Site 3	Okana Stream	Native bush surrounded by sheep and beef pasture and abandoned grapevines

Figure 3.25: Kaituna Valley, (A) site 1 through farming area, and (B) site 2 Kaituna Valley scenic reserve.

3.3.3.5 Lake Hood

3.3.3.5.1 *Phytophthora* isolated from Lake Hood

Lake Hood, which is a man-made recreational lake (Figure 3.26 A), lies alongside the Ashburton River (sampling site was 3.7 km away from Ashburton River site 6; Figure 3.26 B). A total of seven *Phytophthora* isolates were recovered from two *Phytophthora* spp., being *Ph. lacustris* (six isolates) and *Ph. chlamydospora* x *Ph. amnicola* hybrid (one isolate).

3.3.3.5.2 Description of land use type in Lake Hood water sampling site

Lake Hood is a man-made recreational lake, surrounded by grass, bushes and trees, with a nearby dairy farm and pasture.

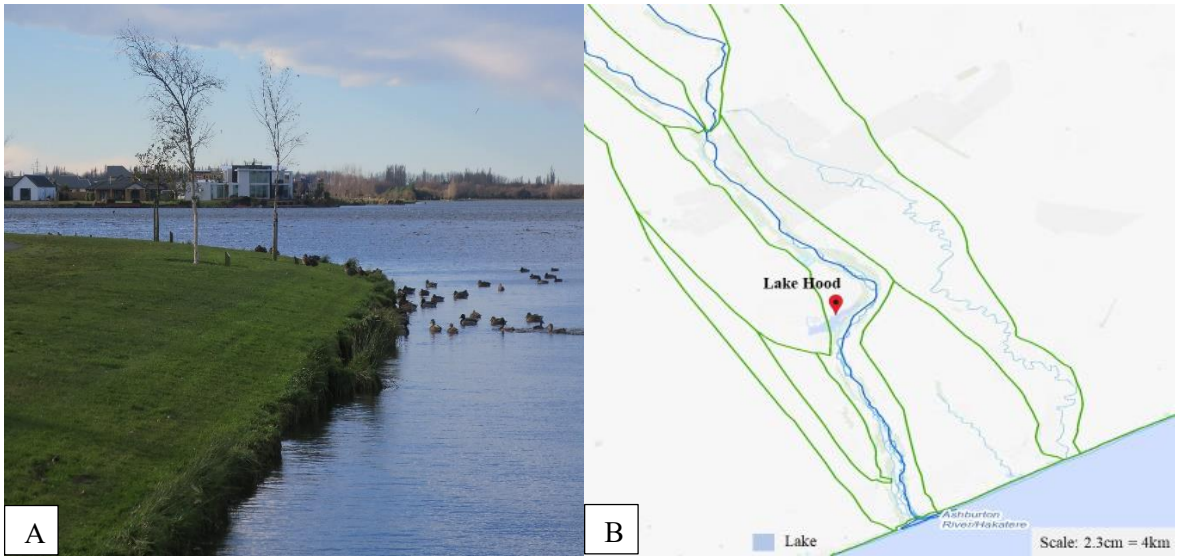

Figure 3.26: Water sampling of one site in Lake Hood, (A) water sampling area, and (B) location of Lake Hood along side the Ashburton River, Source: Land Information New Zealand (2019).

3.3.3.6 Halswell River

3.3.3.6.1 *Phytophthora* isolated from the Halswell River

Three sites were sampled from the Halswell River (Figure 3.27) with site 1 and site 2 being the same sites as sampled for Objective 1 (Chapter 2). All three sites were on the Canterbury Plains with site 1 being from a reserve in Tai Tapu village, site 2 on the outskirts of the village (2.2 km away from site 1) and site 3 (11.2 km away from site 1 and 1.0 km away from Lake Ellesmere) before the river emptied into Lake Ellesmere. The lowest number of *Phytophthora* isolates (five isolates) was recovered from site 2 representing two *Phytophthora* spp., being *Ph. lacustris* (three isolates) and *Ph. gonapodyides* (two isolates). From site 1, nine *Phytophthora* isolates were recovered representing six *Phytophthora* spp., including *Ph. lacustris* (two isolates), *Ph. amnicola* (one isolate), *Ph. chlamydospora* (one isolate), *Ph. chlamydospora* x *Ph. amnicola* hybrid (two isolates), *Ph. chlamydospora* x *Ph. thermophila* hybrid (one isolate) and *Ph. thermophila x amnicola* hybrid (two isolates). The highest number of *Phytophthora* isolates (14 isolates) were recovered from site 3 representing four *Phytophthora* spp., being *Ph. lacustris* (four isolates), *Ph. gonapodyides* (six isolates), *Ph. chlamydospora* x *Ph. amnicola* hybrid (three isolates) and *Ph. thermophila x amnicola* hybrid (one isolate).

3.3.3.6.2 Description of land use type in Halswell River water sampling site

The three sites in the Halswell River were also surrounded by sheep, dairy and pasture (Table 3.6). Site 1 had variable vegetation due to different plants grown in the gardens in the Tai Tapu village.

Figure 3.27: Water sampling of three sites in the Halswell River, Source: Land Information New Zealand (2019).

Table 3.6: Land use types for the three sites sampled from the Halswell River.

River sites	Area Description	Land use
Site 1	Residential and farming area	Flows through Tai Tapu village, gardens, willow trees and bushes,
Site 2	Residential and farming area	Flows though sheep, dairy and pasture, surrounded by weeds
Site 3	Farming area	Passes through dairy farm and pasture

3.3.4 Abiotic factors affecting *Phytophthora* spp. in the waterways

There was no correlation between the number of *Phytophthora* spp. recovered and the five abiotic factors tested; salinity, pH, ammonium, nitrate and water temperature. (Table 3.7). Recovery of *Phytophthora* spp. was not affected by the abiotic factors over the range measured in the waterways being, salinity (range 0-0.2ppt), pH (range 6.7-7.8), ammonium (range 0.1-0.23 mg/L; Appendix A.3.3, Table A6), nitrate (range 0.03-7.36 mg/L; NO_3^-) and water temperature (range 4^{o}C -12^{o}C).

Table 3.7: Pearson's correlations of the number of *Phytophthora* spp. recovered with the different water abiotic parameters measured (salinity, pH, water temperature, ammonium, and nitrate) and their corresponding P-values.

Water parameters	Pearson's r Species	P value Species
Salinity (ppt)	0.2977	0.0095
pH	0.2789	0.0154
Water temperature (^{o}C)	0.0658	0.5745
Ammonium (NH_4; mg/L)	0.4304	0.0001
Nitrate (NO_3; mg/L)	0.0231	0.8439

3.3.5 Abiotic factors affecting *Phytophthora* isolates in the waterways

The was no correlation between the number of *Phytophthora* isolates recovered and the five abiotic factors over the range measured in the (Table 3.8) salinity (range 0-0.2ppt; Appendix A.3.3; Table A6), ammonium (range 0.1-0.23 mg/L; Appendix A.3.3; Table A7), water temperature (range 4^{o}C - 12^{o}C), pH (range 6.7-7.8) and nitrate (range 0.03-7.36 mg/L).

Table 3.8: Pearson's correlations of the number of *Phytophthora* isolates recovered with the different water abiotic parameters measured (salinity, pH, water temperature, ammonium, and nitrate) and their corresponding P-values.

Water parameters	Pearson's r Isolates	P value Isolates
Salinity (ppt)	0.2925	0.0109
pH	0.1765	0.1298
Water temperature	0.0213	0.8561
Nitrogen_NH_4 (mg/L)	0.4663	≤0.0001
Nitrogen_NO_3 (mg/L)	-0.0426	0.7167

3.3.6 Difference in *Phytophthora* spp. isolated from the Halswell River in summer and autumn

There was no difference in the number of *Phytophthora* spp. recovered from the two sites in the Halswell River in summer (February; Chapter 2) and autumn (May; P=0.25; Appendix A.3.3; Table A7). Therefore, site was removed from the analysis which showed that bait type (P=0.04) and season (P=0.04) had an effect on the number *Phytophthora* spp. recovered, while there was no significant interaction between bait and season on the number of *Phytophthora* spp. (P=0.24; Appendix A.3.3; Table A8). The number of *Phytophthora* spp. obtained was also evaluated with salinity and pH as the covariate. Salinity did not affect the *Phytophthora* spp. isolated whilst bait type (P=0.04) and season (P=0.04; Appendix A.3.3; Table A9) having an effect on the number of *Phytophthora* spp. recovered. However, when pH was accounted for, there was no effect of bait type (P=0.07) and season (P=0.14; Appendix A.3.3; Table A10) on the number of *Phytophthora* spp. recovered in the two sites in summer and autumn indicating that pH played a role in *Phytophthora* spp. recovery. Irrespective of pH *Phytophthora* spp. were recovered using *R. arboreum* as a bait however, pH seemed to affect the efficiency of *Ce. deodara* and *Pi. radiata* baits in recovering *Phytophthora* (Table 3.9).

Table 3.9: Mean number of *Phytophthora* spp. recovered on *R. arboreum*, *Ce. deodara* and *Pi. radiata* used to bait water samples at different pH levels.

	pH levels			
Bait type	**6.9**	**7.5**	**7.6**	**7.9**
Ce. deodara	0.00	0.00	2.00	2.00
Pi. radiata	1.00	0.00	0.00	1.00
R. arboreum	2.00	1.00	2.00	4.00

Observations on the *Phytophthora* spp. recovered in the two seasons showed variations in the two sites in summer and autumn. *Phytophthora lacustris* was found both in summer and autumn, *Phytophthora* sp. LS-2018c strain CL 181 was only found in site 1 and site 2 in summer and *Ph. chlamydospora* and *Ph. gonapodyides* were only recovered in autumn. Two hybrids were obtained i.e., *Phytophthora thermophila x amnicola* hybrid isolates were recovered from both summer and autumn, while *Ph. chlamydospora* x *Ph. amnicola* hybrid was only recovered in autumn (Figure 3.28).

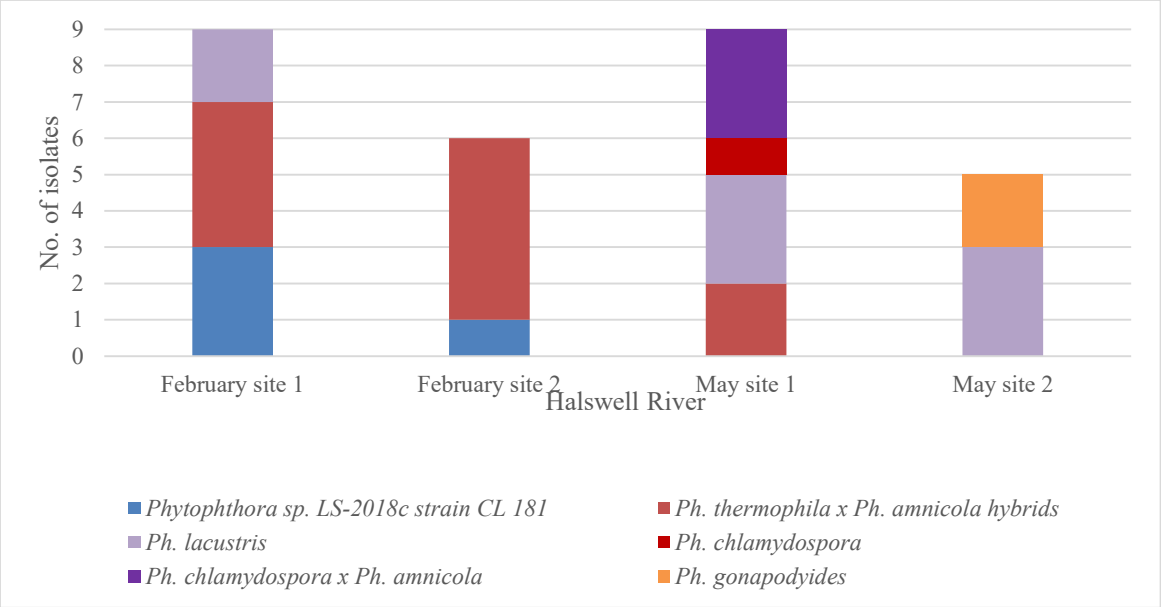

Figure 3.28: *Phytophthora* species recovered from site 1 and site 2 of the Halswell River during the summer (February) and autumn (May) of 2018 using the laboratory baiting method on three bait types (*R., Pi. radiata and Ce. deodara*).

When the effect of bait type and season on the number of *Phytophthora* isolates obtained from the two Halswell River site was evaluated, bait type (P=0.003) significantly affected the number of isolates while the season (P=1.00) or the interaction between bait and season (P=0.20; Appendix A.3.3; Table A11) did not significantly affect the number of *Phytophthora* isolates obtained. *Rhododendron arboreum* recovered more *Phytophthora* isolates than *Pi. radiata* and *Ce. deodara* (Figure 3.29).

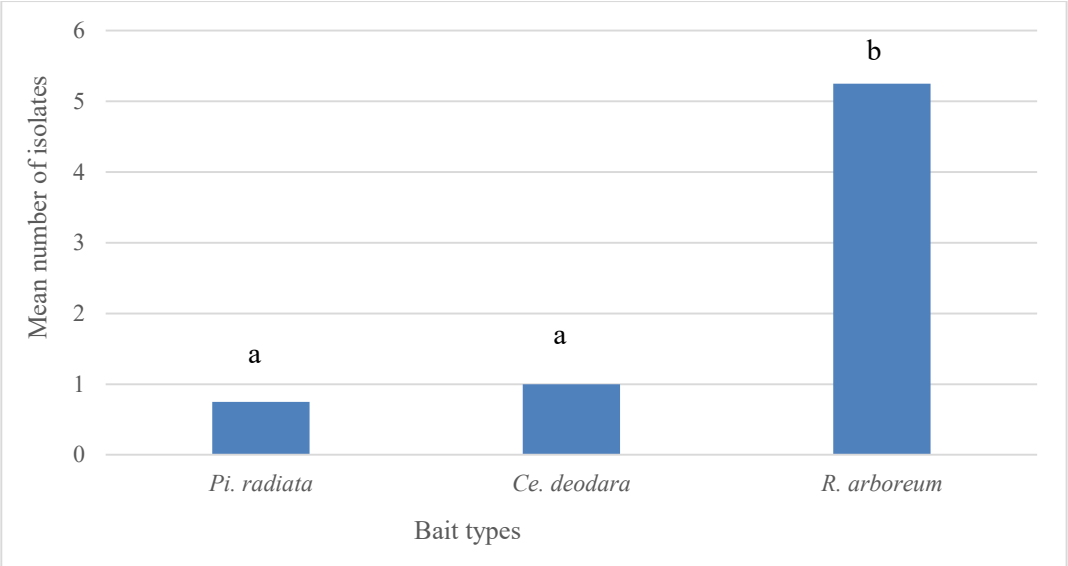

Figure 3.29: The mean number of *Phytophthora* spp. isolate recovered from site 1 and site 2 of the Halswell River during the summer (February) and autumn (May) of 2018 on three baits, *R. arboreum, Pi. radiata and Ce. deodara.* Bars followed by different letters are significantly different at P=0.05.

When the number of isolates recovered were analysed taking pH into account bait type still significantly affected the number of isolates (P=0.007; Appendix A.3.3; Table A12) with *R. arboreum* recovering more *Phytophthora* isolates than *Pi. radiata* and *Ce. deodara* (Appendix A.3.3; Table A13). When all the parameters (pH, salinity, water temperature) measured were accounted for, bait type (P=0.003; Appendix A.3.3; Table A14) had the greatest effect on the number of *Phytophthora* isolated recovered with *R. arboreum* being best at recovering *Phytophthora* isolates (Appendix A.3.3; Table A15).

Comparison of the water parameters measured, showed that pH and ammonium nitrogen levels of the water samples fluctuated in the two sites between the summer and autumn sampling (Table 3.10). There was no difference in the salinity levels. Despite the changes in season, the water temperature remained constant with only minor variation in the nitrate nitrogen levels between seasons in the two sites.

Table 3.10: The number of *Phytophthora* species and isolates recovered and the salinity (ppt), pH, water temperature (°C), ammonium nitrogen (NH_4-N; mg/L) and nitrate nitrogen levels (NO_3-N; mg/L) from two sites in the Halswell River during summer (February 2018) and autumn (May 2018) water sampling.

Sampling date	Site	No. of *Phytophthora* spp.	No. of isolates	Salinity (ppt)	pH	Water temp (°C)	NH_4-N (mg/L)	NO_3^-N (mg/L)
February 2018	1	3	13	0.1	6.9	12	0.08	2.71
February 2018	2	5	18	0.1	7.5	10	0.11	2.29
May 2018	1	6	9	0.1	7.9	10	0.1	2.84
May 2018	2	2	5	0.1	7.6	11	0.14	2.88

3.3.7 Evaluation of the leaf baits

When the sites and bait type that did not recover any *Phytophthora* were removed from the analysis, bait type was found to be the only factor affecting the number of *Phytophthora* spp. (P=0.004, Appendix A.3.3; Table A4) and the number of *Phytophthora* isolates (P=0.01, Appendix A.3.3; Table A5) recovered from waterways (Section 3.3.3 and 3.3.6). *Rhododendron arboreum* was the best bait in isolating the highest number of *Phytophthora* isolates.

When the sites and bait type that did not recover any *Phytophthora* were removed from the analysis the unbalanced data prevented further analysis to identify the best bait. Therefore, only the sites that did not recover any *Phytophthora* were removed from the analysis. When all of the 22 sites that recovered *Phytophthora* were analysed, there was a significant effect of bait on the number of *Phytophthora* isolates recovered ($P < 0.001$; Appendix 3.3; Table A16). Multiple comparison of the mean using Tukey's test revealed that all the3 baits were significantly different from each other, with *R. arboreum* being highly significantly different from *Pi. radiata* ($P < 0.001$) and *Ce. deodara* (P=0.01; Table 3.11) in the number of *Phytophthora* isolates recovered. *Rhododendron arboreum* (mean=1.8) recovered the highest number of *Phytophthora* isolates followed by *Ce. deodara* (mean=1.3) and *Pi. radiata* (mean=1.2).

Table 3.11: Tukey's honestly significant difference (HSD) test for comparison of the number of *Phytophthora* isolates recovered by the different bait types.

Linear hypothesis	Estimate std.	Error	t value	P value
Pi. radiata - *Ce. deodara* == 0	-0.3636	0.1526	-2.384	0.0474 *
R. arboreum - *Ce. deodara* == 0	0.4697	0.1526	3.079	0.0066 **
R. arboreum - *Pi. radiata* == 0	0.8333	0.1526	5.462	0.0001 ***

* significantly different (P≤0.05), ** highly significantly different (P≤0.005), *** highly significantly different (P≤0.0005).

There was a significant difference (P < 0.001; Appendix A.3.3; Table A17) in the number of *Phytophthora* spp. isolated from the 3 leaf bait types and Tukey's test revealed that all the 3 baits differed significantly (P<0.05; Table 3.12) from each other. A higher number of *Phytophthora* spp. was recovered on *R. arboreum* (mean = 1.0) followed by *Ce. deodara* (mean = 0.6) and *Pi. radiata* (mean = 0.3).

Table 3.12: Tukey's honestly significant difference (HSD) test for comparison of the number of *Phytophthora* spp. recovered by the different bait types

Linear hypothesis	Estimate std.	Error	t value	P value
Pi. radiata - *Ce.deodara* == 0	-0.3030	0.1238	-2.447	0.0403 *
R. arboreum - *Ce.deodara* == 0	0.3788	0.1238	3.059	0.0071 **
R. arboreum - *Pi. radiata* == 0	0.6818	0.1238	5.506	<0.0001***

* significantly different (P≤0.05), ** highly significantly different (P≤0.005), *** highly significantly different (P≤0.0005).

Looking at the individual baits and the *Phytophthora* species recovered; *Rhododendron arboreum* isolated all eight *Phytophthora* spp. (*Phytophthora lacustris, Ph. gonapodyides, Ph. chlamydospora* x *Ph. amnicola* hybrid, *Ph. thermophila x amnicola* hybrid, *Ph. chlamydospora* x *Ph. thermophila* hybrid, *Ph. chlamydospora*, *Ph. amnicola,* and *Ph. bilorbang)*; followed by *Pi. radiata* which isolated five species (*Phytophthora lacustris, Ph. gonapodyides, Ph. chlamydospora* x *Ph. amnicola* hybrid, *Ph. thermophila x amnicola* hybrid, *Ph. chlamydospora* x *Ph. thermophila* hybrid*)*; and *Ce. deodara*

isolated four species (*Phytophthora lacustris, Ph. gonapodyides, Ph. chlamydospora* x *Ph. amnicola* hybrid, *Ph. chlamydospora*; Figure 3.30).

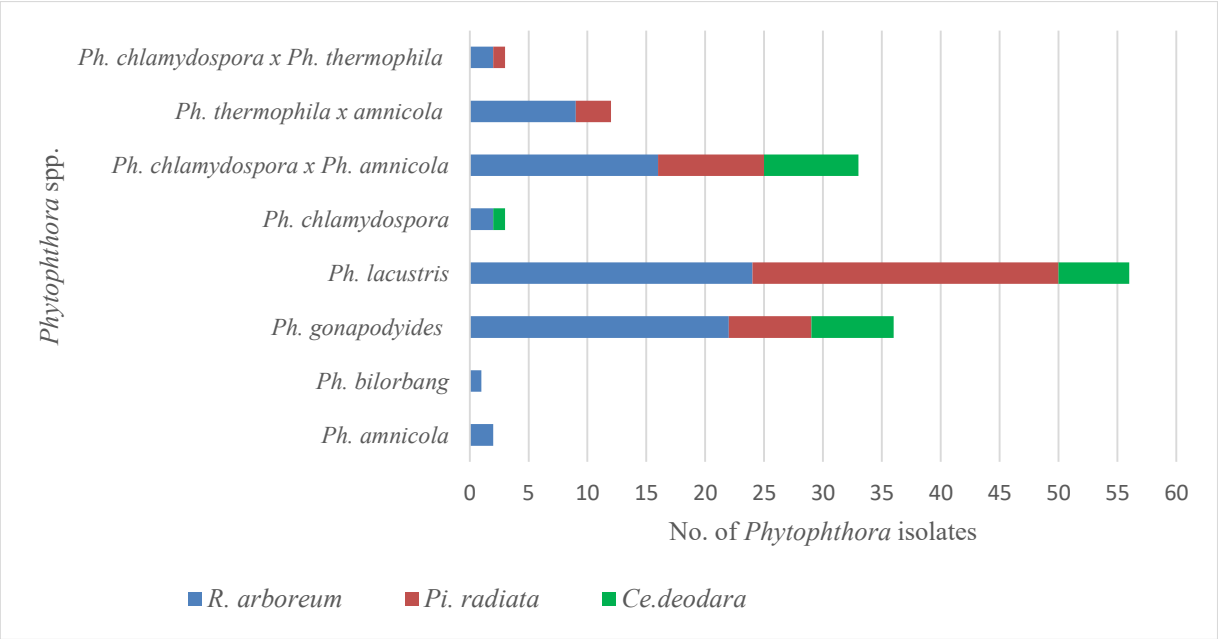

Figure 3.30: The number of isolates of the different *Phytophthora* spp. isolated by the three leaf baits *Rhododendron arboreum, Pinus radiata* and *Cedrus deodara*, used to bait from water samples taken from 25 sites in Canterbury waterways.

3.4 Discussion

3.4.1 Diversity of *Phytophthora* spp. from the 6 waterways in Canterbury

The extensive *Phytophthora* study carried out in autumn (May 2018) in the six waterways recovered 8 *Phytophthora* spp. which were found to be widespread, rather than localised, in their distribution. *Phytophthora lacustris* was the most commonly recovered spp. recovered from 19 out of the 25 sites, followed by *Ph. gonapodyides* (recovered from 14 sites), *Ph. chlamydospora* x *Ph. amnicola* hybrid (recovered from 15 sites), *Ph. thermophila x amnicola* hybrid (recovered from eight sites), *Ph. chlamydospora* x *Ph. thermophila* hybrid (recovered from three sites), *Ph. chlamydospora* (recovered from three sites), *Ph. amnicola* (recovered from three sites) and *Ph. bilorbang* (one site). All these species belong to clade 6 which is similar to what other studies have isolated commonly from waterways as discussed in Chapter 2 Section 2.4.3 (Hüberli *et al.*, 2013; Randall, 2011; Reeser *et al.*, 2011; Stamler *et al.*, 2016a). The three *Phytophthora* hybrids (*Ph. chlamydospora* x *Ph. amnicola, Ph. thermophila x amnicola, Ph. chlamydospora* x *Ph. thermophila*), *Ph. lacustris* and *Ph. gonapodyides* were also recovered in Chapter 2 summer (February 2018) baiting, however, the autumn baiting reported in this chapter also isolated the parent strains *Ph. amnicola* and *Ph. chlamydospora*, and Ph. bilorbang which were not recovered in the summer baiting (Chapter 2). *Phytophthora* spp. isolated both in the summer and autumn baiting indicate that these species are common in the areas surrounding the waterways sampled in the Canterbury region.

Phytophthora bilorbang isolated from Selwyn River site 1 has been previously isolated in Auckland from roots and soils of *Fraxinus angustifolia* (Ash) (Manaaki Whenua Landcare Research, 2019b, 2019d). *Phytophthora bilorbang,* which belongs to clade 6 sub-clade II, was first isolated in Australia from declining *Rubus anglocandicans* (European blackberry) and has been isolated from river catchments and river banks (Aghighi *et al.*, 2012; Kang, 2016b). *Phytophthora bilorbang* has also been isolated from oak stands in Europe and appears to be a common species in natural environment (Aghighi *et al.*, 2012; Jung *et al.*, 2018b). The ITS sequence of this isolate identified it as *Phytophthora* taxon oaksoil while the *coxI* gene identified it as *Ph. bilorbang.* This was similar to the findings of Kang (2016b) which found the ITS sequence of *Ph. bilorbang* to be identical to that of *Phytophthora* taxon oaksoil. Nonetheless these two species are different in morphology as *Phytophthora* taxon oaksoil is homothallic while *Ph. bilorbang* is sexually sterile (Kang, 2016b). Isolation of the *Ph. bilorbang* isolate from Selwyn site 1 (camping site/ reserve) could be due to a nearby diseased oak tree, ash tree or European blackberry with low levels of zoospores present in the river at the time of water sampling. Identifying the distribution of *Ph. bilorbang* in New Zealand is important as this species has previously been identified as pathogenic to more than one plant species (Jung *et al.*, 2018b).

3.4.2 Difference in *Phytophthora* recovery and land use

When the data of sites and bait type that did not recover any *Phytophthora,* spp. were removed from the analysis, the site was found to have no effect on the number of *Phytophthora* isolates and species recovered, with only bait type affecting *Phytophthora* recovery. However, when the data of sites with no *Phytophthora* recovery (Ashburton site 4, Selwyn site 7 and site 8) was removed from the analysis, difference in land use (at 90% confidence) is evident and had an effect on the *Phytophthora* species diversity isolated from 4 of the 25 sites sampled. The difference in *Phytophthora* diversity was seen between Kaituna Valley site 3 compared with Prices Valley site 3, Ashburton site 3 and Selwyn site 2, and mostly due to the high species diversity (14 isolates from 5 species) recovered from the Kaituna Valley site 3 compared with only 1 isolate from each of the other sites. The 25 river sites sampled varied in land use and location, however very few sites affected the *Phytophthora* diversity recovered. This could be due to the limited differences in the plant diversity close to the river in many of these sites. A number of the sites sampled were from braided rivers (Ashburton River sites 1,2 ,5 and 6, Selwyn River sites 2, 7 and 8) which had large distances (large areas of bushes/scrub and bare stony land) between the land use and the river bank acting as a buffer zone potentially preventing the *Phytophthora* from reaching the waterways. Further, similar riparian plants (willow, poplars and other bushes) were found on the river banks for many of the other sites sampled for the Selwyn River (sites 1, 3, 4, 5 and 6) which may have affected the *Phytophthora* spp. diversity. In contrast, rivers in Banks Peninsula and the Ashburton River sites in the Canterbury foothills ran directly through the land use type and the *Phytophthora* isolated from these areas potentially give a better representation of the species present. Redondo *et al.* (2018) identified environment filtering as a major factor affecting the species diversity in the environment, with stronger environmental filtering occurring under harsh climatic conditions. The presence of large areas of bush and bare rocky ground on the braided river banks is likely to present harsh conditions for zoospore survival preventing the *Phytophthora* spp. present in the land area from entering the waterways. Therefore, the species recovered along the braided river may not be a true representation of the *Phytophthora* spp. present in the surrounding land area.

Phytophthora spp. diversity in the Canterbury waterways is likely to be favoured by different types of land use. In Prices Valley, sites 1 and 2 had the same land use type (regenerating native bush with sheep grazing) and recovered similar species i.e., site 1 recovered (three species) *Ph. lacustris, Ph. gonapodyides* and *Ph. chlamydospora* x *Ph. amnicola* hybrid, while site 2 (three species) recovered *Ph. lacustris*, *Ph. chlamydospora* x *Ph. amnicola* hybrid and *Ph. chlamydospora* x *Ph. thermophila* hybrid. Prices Valley site 3 had old protected bush recovering only one isolate of *Ph. lacustris*. Sites 1, 2 and 3 flowed into Prices Valley site 4, which had a different land use from other three sites i.e., tree/bush, sheep and beef farming recovering the highest number of *Phytophthora* spp. (four species), *Ph. lacustris, Ph. gonapodyides*, *Ph. chlamydospora* x *Ph. amnicola* hybrid and *Ph. chlamydospora* x *Ph. thermophila* hybrid. Low *Phytophthora* species diversity was obtained from site 3 which had

minimal anthropogenic activity (site 3 old protected bush) with higher *Phytophthora* spp. diversity obtained around replanted bush (sites 1 and 2) and agricultural land use (site 4). Higher *Phytophthora* spp. diversity in agricultural areas and replanted bush can be a result of changes in the environment influenced by plant diversity through anthropogenic activities or introduction of *Phytophthora* spp. through infected planting material. The effect of land use type on *Phytophthora* spp. diversity in waterways is supported by the findings of Redondo *et al.* (2018) where agricultural areas had higher levels of *Phytophthora* spp. than in natural forests with the distribution of pathogenic *Phytophthora* spp. being linked to plant diversity. Lewis (2018) also identified land use effect on *Phytophthora* spp. where early establishment of *Ph. agathidicida* in soil was favoured in pine and pasture soils. The diversity of *Phytophthora* species isolated from Kaituna Valley River was similar to that from Prices Valley River. However, higher *Phytophthora* diversity (five species) and isolate numbers (14 isolates) were obtained from a stream (Okana stream, Kaituna site 3) running into the Kaituna River than the other two river sites. Site 1 recovered three isolates representing three *Phytophthora* spp., while site 2 recovered five isolates representing four *Phytophthora* spp. Recovery of higher *Phytophthora* diversity and isolate numbers could be due to less dilution of *Phytophthora* zoospores in smaller streams compared to larger the river sites. Reeser *et al.* (2011) suggested from their results that *Phytophthora* spp. sporulate constantly, however *Phytophthora* spp. isolation is affected by dilution of the inoculum in the waterways due to the water levels. Redondo *et al.* (2018) reported that 60% of the *Phytophthora* spp. that were isolated from soil were also isolated from stream baiting in the same sites, with a possibility of species becoming diluted once they enter the stream. Further *Phytophthora* surveys in waterways should be focused on smaller streams located close to land use types that have higher environmental disturbances to provide a more accurate indication of the *Phytophthora* species in the surrounding land areas.

Larger differences in *Phytophthora* spp. diversity is evident between regions rather than within regions. Except for *Phytophthora* sp. LS-2018c strain CL 181isolated in summer (Section 2.3.3) in Halswell River sites 1 and 2, similar *Phytophthora* spp. were recovered in summer and autumn baiting of all the waterways in Canterbury. However, Randall (2011) recovered only two of the same *Phytophthora* spp. (*Ph. gonapodyides* and *Ph. chlamydospora*) in Auckland, with all other species recovered in the Auckland study not found in the current Canterbury baiting. The difference in *Phytophthora* recovery between Auckland and Canterbury could be due to differences in the climate between the two regions. Auckland has warm humid summers (maximum daytime temperature range from 22°C to 26°C seldom exceeding 30°C) and mild winters (maximum daytime temperature range from 12°C to 17°C) (Mackintosh, 2019). Canterbury summers are hotter than Auckland (maximum daytime temperature ranges from 18°C to 26°C, and often exceeds 30°C) and cold winters with frequent frost (daytime maximum air temperatures range from 7°C to 14°C) (Mackintosh, 2019). The Canterbury region is overall drier and has long dry spells especially in the summer and a low annual rainfall of 500-750 mm, while Auckland's climate is wetter with an average annual rainfall of 1000-1250 mm (Mackintosh, 2019). A *Phytophthora* diversity study in the waterways of Western Australia

also identified distinct regional differences in the *Phytophthora* spp. recovered and linked it to geographic differences and variation in season (Hüberli *et al.*, 2013). Both the plant diversity and the microbial community of the region is affected by environmental filtering processes (Redondo *et al.*, 2018). According to Redondo *et al.* (2018) the differences in climatic conditions triggers the environmental filtering process that selects for certain traits and subsequently against some species determining the regional species diversity. Difference in *Phytophthora* spp. diversity is based on the ability of the species to adapt to different environments. *Phytophthora* spp. diversity is also dependant on the presence of host plants to infect. Varying physiological traits (sexual and /or asexual reproductive structures i.e., oospores, chlamydospores, zoospores and hyphal swellings) amongst *Phytophthora* spp. enable them to thrive in different environmental conditions (Redondo *et al.*, 2018). Species which produce chlamydospores or hyphal swelling are more likely to be found in colder and drier regions as these structures enable *Phytophthora* spp. to tolerate low temperatures and drought conditions (Redondo *et al.*, 2018). In the current study, species producing chlamydospores, *Ph. chlamydospora* and *Ph. thermophila,* with *Ph. thermophila* also reported to produce hyphal swellings (Jung *et al.*, 2011; Kang, 2016a) and non-chlamydospore producing *Ph. gonapodyides* were commonly found in the Canterbury region. While in Auckland Randall (2011) also found *Ph. chlamydospora* and *Ph. gonapodyides* as well as and *Ph. multivora* which does not produce chlamydospore or hyphal swellings (Kang, 2016a) to be common. This indicates that the difference in the *Phytophthora* spp. in Auckland and Canterbury regions is not limited to the ability of these species to produce chlamydospores and hyphal swellings, and the differences in the species diversity is probably due to environmental filtering of other traits, and likely to include the difference in plant communities in the surrounding landscape. Regions with varying climatic conditions are likely to have more differences in *Phytophthora* spp. diversity and should be targeted when surveying larger areas for *Phytophthora* diversity studies.

3.4.3 Abiotic factors affecting *Phytophthora* in waterways

In this study the abiotic factors tested being salinity (range 0-0.2ppt), pH (range 6.7-7.8), ammonium (range 0.1-0.23 mg/L), nitrate (range 0.03-7.36 mg/L) and water temperature (range 4^{o}C -12^{o}C) were found to have no effect on the *Phytophthora* spp. diversity and the total number of *Phytophthora* isolates recovered. However, Redondo *et al.* (2018) found temperature and water chemistry (pH, conductivity, total organic carbon, and total nitrogen) were strong environmental drivers for the diversity of aquatic *Phytophthora* spp. In the study by Redondo *et al.* (2018), water pH and total nitrogen had a positive correlation with the number of *Phytophthora* spp., while comparatively salinity had a negative correlation with the number of *Phytophthora* spp. in waterways and lower temperatures favoured *Phytophthora* spp. diversity. The lack of a significant relationship between *Phytophthora* diversity and abiotic factors in this study could be due to the small number of sites studied and little variation in the water parameters tested. Redondo *et al.* (2018) findings were based on 96 sites in 16

rivers sampled over 2 year that had varying land use categorised as urban, agricultural and forest. Difference in the water chemistry between environments was linked to the different aquatic *Phytophthora* communities recovered from river sites located in cities, agricultural fields and in forests (Redondo, 2018).

The current study used 25 sites with little variation in land use type such as braided rivers (Ashburton (sties 1,2,5 and 6) and Selwyn River (sites 2, 7, and 8)) with large areas of bush and bare stony land separating the land use and river. Five sites in the Selwyn River (sites 1,3,4,5 and 6) had similar land use with large amount of bush and willow trees close to the river. Two of the Ashburton River sites were associated with native bush and the seven sites in Banks Peninsula had native bush or agricultural land use. Similarity in land use type resulted in little variation in the levels of the abiotic factors measured in the 25 sites which showed no significant relationship with the *Phytophthora* spp. diversity. Since land use affects the quantity and quality of water nutrients, which influences water aquatic *Phytophthora* communities (Redondo *et al.*, 2018) water baiting should include more diverse sites in terms of land use, especially urban areas which are likely to have different water chemistry with abiotic factors assessed over a larger number of sites.

3.4.4 Seasonal difference in *Phytophthora* isolated from Halswell River

The difference in the number of *Phytophthora* isolates recovered in summer and autumn depended on the bait type rather than the season. However, observations on the *Phytophthora* spp. recovered in the two seasons showed variations in the two sites in summer (three species being, *Phytophthora* sp. LS-2018c strain CL 181, *Ph. thermophila x amnicola* hybrid and *Ph. lacustris*) and autumn (five species i.e., *Ph. thermophila x amnicola* hybrid, *Ph. chlamydospora* x *Ph. amnicola* hybrid *Ph. lacustris, Ph. chlamydospora* and *Ph. gonapodyides*). Seasonal differences in *Phytophthora* spp. recovered from waterways can be due to changes in water chemistry, which was identified by Redondo *et al.* (2018) as a strong environmental driver for the diversity of aquatic *Phytophthora* , with decreasing water temperature and precipitation favouring *Phytophthora* diversity. *Phytophthora* spp. that were pathogens on hosts located out of the riparian zone were detected in waterways during seasons (spring, summer and autumn) that had lowest rainfall and greatest temperature (Randall, 2011). According to Wielgoss *et al.* (2009), identification of a single factor responsible for changes in the natural ecosystem due to seasons is difficult as seasonality is a net result of complex interactions between several factors. Therefore, this could explain the difference in *Phytophthora* spp. diversity seen in summer and autumn in the Halswell River (site 1 and 2) despite small fluctuations observed in the water parameters measured (pH, temperature, nitrogen and salinity). In this study, there was no seasonal difference in the number of *Phytophthora* isolates recovered in summer and autumn. While Reeser *et al.* (2011) found that the highest number of *Phytophthora* colonies (isolates) were recovered in summer, followed by autumn, winter and spring. Lack of difference in the number of isolates can be due to the low number of sites (two sites) and seasons (two seasons) used in this study. Randall (2011)

also identified seasonal differences in *Phytophthora* spp. recovery and abundance with the highest number of isolates recovered in summer and autumn, followed by spring and winter in the Auckland survey. Further *Phytophthora* surveys in waterways should focus on baiting across all seasons for a consensus on the *Phytophthora* spp. diversity in an area.

3.4.5 Evaluation of the three leaf baits for baiting for *Phytophthora* recovery

Rhododendron arboreum was identified as the best bait that recovered more *Phytophthora* isolates and *Phytophthora* spp. when compared to *Pi. radiata* and *Ce. deodara,* which was probably related to the variability in their efficiency in isolating *Phytophthora* across different water pH. The Halswell River seasonal study (Section 3.4.5) and evaluation of *Phytophthora* recovery from the Canterbury waterways showed that the bait type affected the number of *Phytophthora* isolates and *Phytophthora* spp. recovered. In Chapter 2, *R. arboreum* was identified as the best bait as it isolated higher number of *Phytophthora* isolates, but no difference was seen in the number of *Phytophthora* spp. isolated by the seven bait types. This could be due to the low number of sites surveyed (two sites) in Chapter 2, however this study used 25 sites which showed that the three baits differed in the number of *Phytophthora* spp. and the number of *Phytophthora* isolates they recovered, with *R. arboreum* identified as the best bait isolating the highest number of *Phytophthora* spp. and number of *Phytophthora* isolates. With regards to the species each bait isolated, *R. arboreum* recovered eight *Phytophthora* spp., while *Ce. deodara* isolated five *Phytophthora* spp. and *Pi. radiata* isolated four *Phytophthora* spp. This concurs with the findings of Martin *et al.* (2012) which identified leaves of *Rhododendron* spp. to yield greater diversity of *Phytophthora* spp. and populations. Hüberli *et al.* (2013) also found that *Phytophthora* sp. isolation varied with the different types of plant species used as baits and some *Phytophthora* spp. were attracted to certain baits and not others. Randall (2011) identified *R. arboreum* as the bait in Auckland waterways that gave the greatest number of *Phytophthora* isolates. However, *R. arboreum* was found to be biased towards isolation of certain *Phytophthora* spp. (Randall 2011). This did not appear to be the case in the current study with *R. arboreum* baits, however the *Phytophthora* species recovered by *Ce. deodara* and *Pi. radiata* did differ and may indicate that these two bait types are more selective in the *Phytophthora* species recovered. Previous studies evaluating *Phytophthora* baits have not accounted for the effect of abiotic factors such as pH on the leaf bait performance in recovering *Phytophthora.* Further *Phytophthora* recovery studies from waterways should use *R. arboreum* leaf bait and future evaluation of baits should evaluate the abiotic factors that affect *Phytophthora* isolations.

Rhododendron arboreum was identified as the best bait that recovered more *Phytophthora* isolates and *Phytophthora* spp. when compared to *Pi. radiata* and *Ce. deodara* and was due to the tolerance of the baits to the fluctuating water pH. Halswell River seasonal study (Section 3.4.5) and evaluation of *Phytophthora* recovery from Canterbury waterways showed that the bait type affected the number of *Phytophthora* isolates and *Phytophthora* spp. recovered. In Chapter 2, *R. arboreum* was identified as

the best bait as it isolated higher the number of *Phytophthora* isolates, but no difference was seen in the number of *Phytophthora* spp. isolated on the seven bait types. This can be due to the low number of sites surveyed (two sites) in Chapter 2, however this study used 25 sites which showed that all the three baits had differences in the number of *Phytophthora* spp. and the number of *Phytophthora* isolates recovered with *R. arboreum* was the best bait and isolated the highest number of *Phytophthora* spp. and number of *Phytophthora* isolates. Looking at the species each bait isolated, *R. arboreum* recovered eight *Phytophthora* spp., while *Ce. deodara* isolated five *Phytophthora* spp. and *Pi. radiata* isolated four *Phytophthora* spp. This concurs with Martin *et al.* (2012) findings, which identified leaves of *Rhododendron* spp. to yield greater diversity of *Phytophthora* spp. and populations. Hüberli *et al.* (2013) also found that *Phytophthora* sp. isolation varied with the different types of plant species used as baits and some *Phytophthora* spp. were attracted to certain baits and not others. Randall (2011) identified *R. arboreum* as the bait in Auckland waterways that gave the greatest number of *Phytophthora* isolates. However, *R. arboreum* was found to have biasness towards isolation of certain *Phytophthora* spp. (Randall 2011). This did not appear to be the case in the current study with *R. arboreum* baits, however the *Phytophthora* species recovered by *Ce. deodara* and *Pi. radiata* did differ and may indicate that these two bait types are more selective in the *Phytophthora* species recovered. Previous studies evaluating *Phytophthora* baits have not accounted for the effect of abiotic factors such as pH on the leaf bait performance in recovering *Phytophthora.* Further *Phytophthora* recovery studies from waterways should use *R. arboreum* leaf bait and future evaluation of baits should evaluate the abiotic factors that affect *Phytophthora* isolations.

3.5 Conclusion

This chapter identified the diversity of *Phytophthora* spp. in 25 sites with varying land use types along six Canterbury waterways. Eight clade 6 *Phytophthora* spp. were isolated in this study and found to be widespread rather than localised in their distribution. *Phytophthora lacustris* (56 isolates recovered from 19 of the 25 sites) was the most commonly recovered species. followed by *Ph. gonapodyides* (36 isolates recovered from 14 sites), *Ph. chlamydospora* x *Ph. amnicola* hybrid (33 isolates recovered from 15 sites), *Ph. thermophila x amnicola* hybrid (12 isolates recovered from eight sites), *Ph. chlamydospora* x *Ph. thermophila* hybrid (three isolates recovered from three sites), *Ph. chlamydospora* (three isolates recovered from three sites), *Ph. amnicola* (three isolates recovered from three sites) and *Ph. bilorbang* (one isolate).

The hypothesis of this study was that *Phytophthora* diversity will be affected by land use type and water parameters. However, land use type and abiotic water parameters (water temperature, pH, nitrogen level and salinity level) had no effect on *Phytophthora* spp. diversity. This was probably due the survey consisting of a large number of sites along braided rivers with the land use being separated from the river by large areas mainly consisting of bushes (willow, poplar) and stony, bare areas which

could have affected the *Phytophthora* spp. diversity in these areas. In addition, the areas surveyed in this study were mainly along native and introduced bush and trees and agricultural land with very little difference in the water parameters measured, however, the inclusion of urban areas, as have been included in other studies, may have resulted in differences in *Phytophthora* spp. recovered. Land use type have been found to affect the water nutrient quality and quantity and since water chemistry is known to affect *Phytophthora* spp. diversity, future water surveys for *Phytophthora* spp. diversity should focus on waterways that passed directly beside different land use types with inclusion of urban areas. However, the current study has shown that the *Phytophthora* species associated with Canterbury waterways mainly belong to clade 6 which are considered as being mainly aquatic *Phytophthora* species (Redondo et al., 2018).

Seasonal (summer and autumn baiting) difference in *Phytophthora* diversity was found in the Halswell River (Site 1 and 2) with higher *Phytophthora* number of isolates recovered in summer than in autumn. Seasonal difference in *Phytophthora* species diversity and number of *Phytophthora* isolates recovered have also been found by other studies. However, very little fluctuation was observed in water parameters measured (pH, temperature, nitrogen and salinity). Therefore, seasonal variations in *Phytophthora* spp. diversity is not due to a single factor rather based on a net result of complex interactions between several factors. Therefore, *Phytophthora* baiting across all seasons in waterways should be done to get a true consensus of *Phytophthora* spp. diversity.

This chapter re-examined the three leaf bait types, *R. arboreum, Pi. radiata* and *Ce. deodara*. Results from the larger number of sites (25) showed that there was a difference in the baits, with *R. arboreum* being the best bait which isolated the highest number of *Phytophthora* isolates and *Phytophthora* spp. The water pH affected the *Phytophthora* recovery on leaf baits with *R. arboreum* being more tolerant to the fluctuating water pH than *Pi. radiata* and *Ce. deodara.*

Chapter 4 : Pathogenicity of *Phytophthora* spp. recovered from waterways

4.1 Introduction

Many *Phytophthora* spp. are serious pathogens causing disease in a large variety of crops worldwide. Numerous *Phytophthora* spp. isolated from aquatic environments are hypothesized to be early colonising saprophytes and limited research has been done to evaluate the pathogenicity of these species (Stamler *et al.*, 2016a). *Phytophthora* clade 6 species have a predominant aquatic lifestyle that depend on the production of asexually produced zoospores to colonise plant debris in waterways (Jung *et al.*, 2011). This has led to sexual degeneration (sexual sterility) and without which, the clade 6 *Phytophthora* spp. lose the ability to generate new genotypes. This could give rise to genetic variation in phenotypic characteristics such as virulence, growth rates and wider host range (Jung *et al.*, 2011). However, clade 6 species such as *Ph. chlamydospora* and *Ph. gonapodyides* are known to be pathogenic on native and exotic trees and have a wide host range. As *Phytophthora* isolation from waterways continues to increase, assessing the pathogenicity of the *Phytophthora* spp. recovered is important as some *Phytophthora* spp. may not have been associated with a host before nor their pathogenicity determined (Drenth & Sendall, 2001).

This study aimed to study the pathogenicity of three commonly recovered *Phytophthora* spp. from Canterbury waterways; *Phytophthora* sp. LS-2018c strain CL 181, *Ph. chlamydospora* and *Ph. gonapodyides* (Chapters 2 and 3). The evaluation of four methods to identify a rapid pathogenicity screening method that could be used for future screenings of *Phytophthora* spp. recovered from waterways, was undertaken. Blue lupin (*L. angustifolius)* has been widely used a bait in isolating *Phytophthora* from waterways and is a standardised bait for isolating *Ph. agathidicida* in New Zealand laboratories (Lewis, 2018; Randall, 2011; Reid, 2006; Stamler *et al.*, 2016a). Previous *Phytophthora* pathogenicity tests have used lupin seedling as it grows quickly and produces a thick, white susceptible radicle that is easily assessed for lesions (Lewis, 2018). Sterile water, sterile soil extract solution and vermiculite, which have been previously identified to promote *Phytophthora* sporulation (Drenth & Sendall, 2001; Jeffers, 2015a; Jeffers, 2006) and mycelium growth (Ivors, 2015b), will be used to produced *Phytophthora* inoculum for the pathogenicity assays in this study and as such giving an indication of pathogenicity. Further, pathogenicity tests on seedlings using direct inoculation of roots with agar plugs containing mycelium has been done to test *Ph. parasitica* pathogenicity on tomato and pepper plants (Boix-Ruiz *et al.*, 2017), and will also be tested in this study. The hypothesis of this study is that the test isolates of *Phytophthora* sp. LS-2018c strain CL 181, *Ph. chlamydospora* and *Ph. gonapodyides* are pathogenic on lupin seedlings, irrespective of the screening method used.

4.2 Materials and Method

A series of assays were carried out to identify the pathogenicity of three species of *Phytophthora, Phytophthora* sp. LS-2018c strain CL 181*, Ph. chlamydospora* and *Ph. gonapodyides* spp., which were isolated from waterways in Canterbury. The isolates were tested on blue lupin (*Lupinus angustifolius*) seedlings using four methods for screening the pathogenicity of the three species (Sections 4.2.1, 4.2.2 and 4.2.3).

4.2.1 Pathogenicity test of *Phytophthora* spp. isolates using agar plugs on lupin seedlings grown in sterile water

One isolate each of *Phytophthora* sp. LS-2018c strain CL 181 (isolate T5)*, Ph. chlamydospora* (isolate 292) and *Ph. gonapodyides* (isolate 352) were grown for two weeks on V8 agar (V8A) prior to inoculation of the host plant. Lupin seed was surfaced sterilised using 10% bleach (containing sodium hypochlorite) for 2 min and rinsed for 1 min in sterile deionised water (SDW) (Reid, 2006). Seeds were subsequently soaked for approximately 12 hours in SDW to soften the testa before being planted in pre-moistened sterile vermiculite trays and incubated at 20°C for two to three days in dark, until the radicle was approximately 2 to 3 cm long (Reid, 2006). A single lupin seedling was submerged in a 15 mL Falcon tube containing either sterilised tap or deionised water and a 5 mm mycelial colonised agar plug for one of the isolates. The experiment included a negative control (no agar plug), however no positive control was included. The lupin seedling was held in place with cling wrap and each placed in a test tube rack (Figure 4.1A and B). Five replicates were set up for each isolate and water treatment, arranged in a completely randomised design in a growth cabinet (12 hr light/12 hr dark, at 18°C) and incubated for 4 days (Appendix A.4.1; Table A1). The seedlings were examined for the presence of lesions on day 4 and if none were visible, the incubation was subsequently extended to 7 days. A high humidity inside the growth cabinet was maintained by placing beakers of SDW in the cabinet.

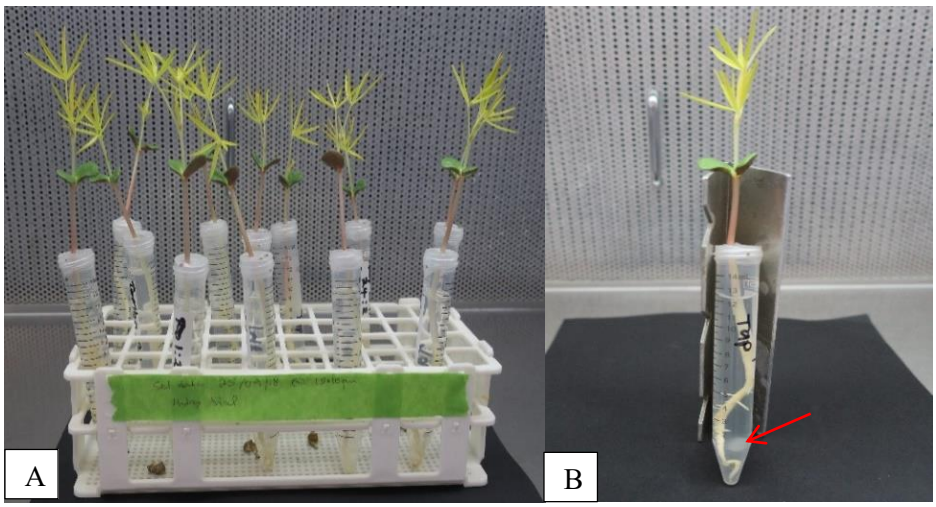

Figure 4.1: Falcon tubes with lupin seedlings (A) arranged in a test tube rack, and (B) with 5 mm agar plug (red arrow).

The degree of root infection was determined by assessing the symptoms and percentage of symptomatic (lesioned) roots for each replicate seedling. The lesions obtained were categorised using a pathogenicity score for each plant (Table 4.1). Samples of lesioned and non-lesioned roots from representative plants per treatment were collected for re-isolation of the causal agent. For re-isolation, the plant material was surface sterilised with 5% bleach for 20 secs, followed by two rinses in sterile water. The roots were air dried in a laminar flow hood on sterile paper prior to cutting the lesioned pieces of root and plating onto a *Phytophthora* selective media (P_5 ARP CMA; Section 2.2.2). The morphology of any colonies that grew from the plant material was compared with the morphology of the *Phytophthora* sp. isolates used for inoculation, to confirm the causal agent.

Table 4.1: Pathogenicity score based on symptoms and percentage of root damage on lupin seedling after four days incubation. Source Beligala (2016).

Score	Description
0	No lesion
1	Visible lesion and minor discoloration on root hairs
2	Extensive lesion and softening
3	Wilting and lesions
4	Dead seedling

4.2.2 Pathogenicity test of *Phytophthora* spp. isolates using agar plugs on lupin seedlings grown in sterile soil extract solution

A pathogenicity test was carried out using one isolate each of *Phytophthora* sp. LS-2018c strain CL 181 (isolate T5), *Ph. chlamydospora* (isolate 292) and *Ph. gonapodyides* (isolate 352) on germinated

lupin seedlings under controlled environmental conditions in a growth cabinet. This experimental set up used sterile soil extract solution (SSES) instead of sterile tap water and deionised water as in Section 4.2.1. Lupin seedlings were germinated as described in Section 4.2.1 and five replicates were set up for the three isolates, positive control *(Ph. cactorum* isolate N17Pla*)* and negative controls (no agar plug) arranged in a randomised block design (Appendix A.4.1; Table A2). *Phytophthora cactorum* was included as a positive control as it is a known plant pathogen (Bonants *et al.*, 2000). *Phytophthora cactorum* isolate N17Pla was sourced from the Lincoln University Plant Pathology culture collection and was recovered by baiting from soil from an apple orchard in Nelson. Four-day old lupin seedlings were transferred individually into SSES in 15 mL Falcon tubes as described in Section 4.2.1. SSES was prepared using 15 g of soil (collected from a flower border at Lincoln University) mixed in 1 litre of deionised water mixed using a magnetic stirrer for four hours (Jeffers, 2015a). The mixture was allowed to settle overnight, followed by filtration using two layers of Whatman #1 filter paper. The solution was sterilized by autoclaving for 15 min at 121°C and stored at 4 °C before being used in the experiment. Lupin seedlings were harvested after 4 days and observations of the lesions and re-isolation of the pathogen was done as described in Section 4.2.1 and if none were visible, the incubation was subsequently extended to 7 days.

4.2.3 Pathogenicity test of *Phytophthora* spp. isolates using agar plugs on lupin seedlings grown on sterile paper towels

In this pathogenicity test one isolate each of *Phytophthora* sp. LS-2018c strain CL 181*, Ph. chlamydospora* and *Ph. gonapodyides* were used to inoculate germinated lupin seedlings which had been germinated on sterile paper towels. Lupin seeds were surface sterilised as described in Section 4.2.1 and soaked overnight prior to being sown. Lupin seeds were germinated in plastic containers lined with two layers of sterile paper towels dampened with SDW, and the lupin seeds placed on the paper towels, spaced out to allow for radicle growth. Two layers of moist paper towels were used to cover the seeds and the container was covered with cling wrap. After 4 days incubation in a growth cabinet (12 hrs light/12 hrs dark, at 18°C) the lupin seedling was inoculated. *Phytophthora* isolates were grown on V8A for 14 days and a 2 mm wide and 8 mm long agar strip containing mycelia was cut and inoculated directly on top of the lupin radicle 1 mm behind the root tip (Robertson, 1968) in the plastic container (Figure 4.2 A and B). Five replicates of each of the three isolates, positive control (*Ph. cactorum* isolate N17Pla) and negative control (no agar plug) were placed in a randomised block design (Appendix A.4.1; Table A2). Lupin seedlings were assessed for symptoms after 4 days (Robertson, 1968) and observations of the lesions and re-isolation of the pathogen was done as described in Section 4.2.1.

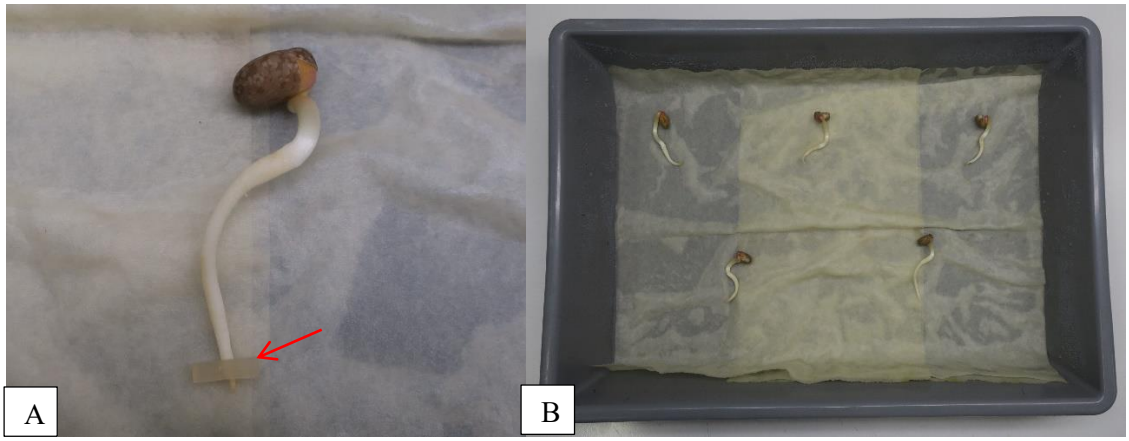

Figure 4.2: Lupin seedling inoculated with *Phytophthora* spp. colonised agar plugs on sterile paper, (A) *Phytophthora* sp. colonised agar strip (red arrow) inoculated on top of the lupin radicle, and (B) seedlings placed in the plastic container prior to inoculation with *Phytophthora* sp.

4.2.4 Pathogenicity test of *Phytophthora* spp. isolates using agar plugs on lupin seedlings grown in sterile vermiculite

This pathogenicity test was done using one isolate each of *Phytophthora* sp. LS-2018c strain CL 181 (isolate T5), *Ph. chlamydospora* (isolate 292) and *Ph. gonapodyides* (isolate 352) on seedlings germinated in sterile vermiculite. Pots containing sterile vermiculite were placed in a saucer (Figure 4.3 A). Seed were directed germinated in the vermiculite (10 mm deep) after surface sterilisation and pre-soaking (Section 4.2.1). Pots were placed in growth a cabinet (12 hrs light/12 hrs dark, at 18°C) for 4 days to allow germination of the lupin seeds. After 4 days, agar plugs from 14-day old *Phytophthora* isolates grown on V8A were used to inoculate the lupin seedlings by placing two mycelial colonised agar plugs in the vermiculite approximately 2 cm away from the seedling at a depth of 2 cm. Five replicates of each of the three isolates, positive control (*Ph. cactorum*) and negative control (no agar plug) was arranged in a randomised block design (Appendix A.4.1; Table A2) in a growth cabinet and incubated for 14 days. All lupin seedlings were kept under continued moist conditions immediately after inoculation by flooding the pots with SDW. Excess water from the pots that drained into the saucers was removed and discarded after 48 hrs (Rigg *et al.*, 2018) followed by watering sparingly daily. The seedlings were harvested 2 weeks after inoculation (Figure 4.3 B), roots were washed, and lesions observed as described in Section 4.2.1. Re-isolation of the pathogen was performed as described in Section 4.2.1.

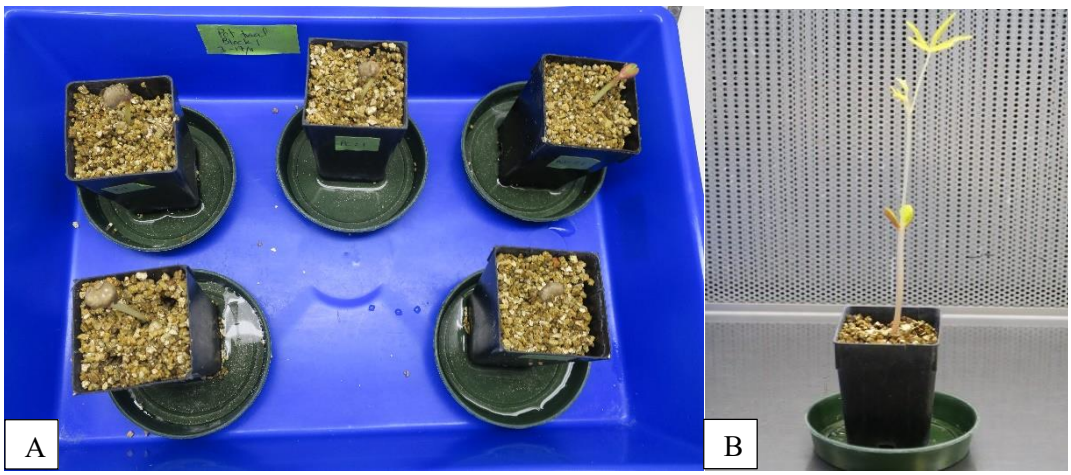

Figure 4.3: Experimental set up for lupin seedling pathogenicity experiment in vermiculite, (A) 4 day-old lupin seedlings in pots containing vermiculite, and (B) seedling growth after 2 weeks.

4.2.5 Data Analysis

Pathogenicity score data was analysed using one-way ANOVA to identify the difference in pathogenicity of the *Phytophthora* spp. isolates. Multiple comparison of the mean pathogenicity score from the *Phytophthora* spp. isolates was done using a Bonferroni test (P=0.05).

4.3 Results

Disease symptoms were only observed on the lupin seedlings in the pathogenicity assay test where lupin seedlings grown on sterile paper towels were inoculated with agar plugs colonised by the *Phytophthora* isolates. Lesions were observed on the lupin seedlings after 4 days (Figure 4.4). Out of the five blocks (Section 4.2.3), data from one block was discarded as lesions were also obtained in the negative control indicating cross contamination had occurred in this block. The causal agent was re-isolated from the seedlings and found to be a *Phytophthora* spp. however, confirmation as to which species was not undertaken. The pathogenicity score obtained from the remaining four blocks was analysed and no difference (P = 0.478; Figure 4.5; Appendix A.4.2; Table A1) was seen between the pathogenicity of *Phytophthora* sp. LS-2018c strain CL 181 isolate T5 (mean score= 1.75), *Ph. chlamydospora* isolate 292 (mean score= 1.5), *Ph. gonapodyides* isolate 352 (mean score= 1) and *Ph. cactorum* isolate N17Pla (positive control; mean score= 1.75) on the lupin seedlings. However, both *Phytophthora* sp. LS-2018c strain CL 181 isolate T5 and *Ph. cactorum* isolate N17Pla (positive control) had a significantly higher pathogenicity score compared with the untreated control, whilst the disease score for *Ph. chlamydospora* isolate 292 and *Ph. gonapodyides* isolate 352 did not differ significantly from the untreated control (Appendix A.4.2; Table A2).

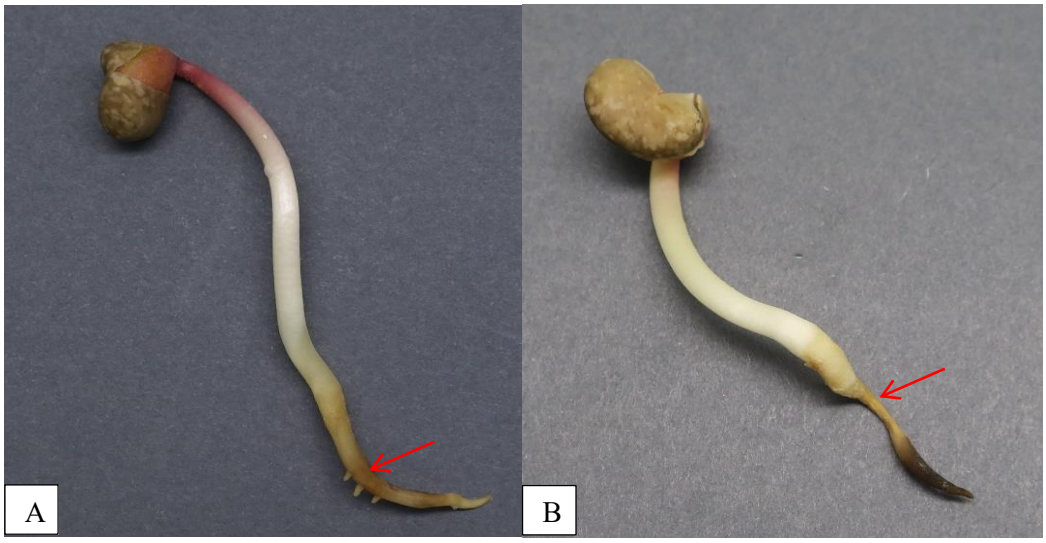

Figure 4.4: Lesions (red arrows) on lupin seedlings inoculated with *Phytophthora* spp., (A) visible lesion and discoloration with inoculation with *Ph. gonapodyides* isolate 352 and (B) lesions and wilting with inoculated with *Phytophthora* sp. LS-2018c strain CL 181 isolate T5.

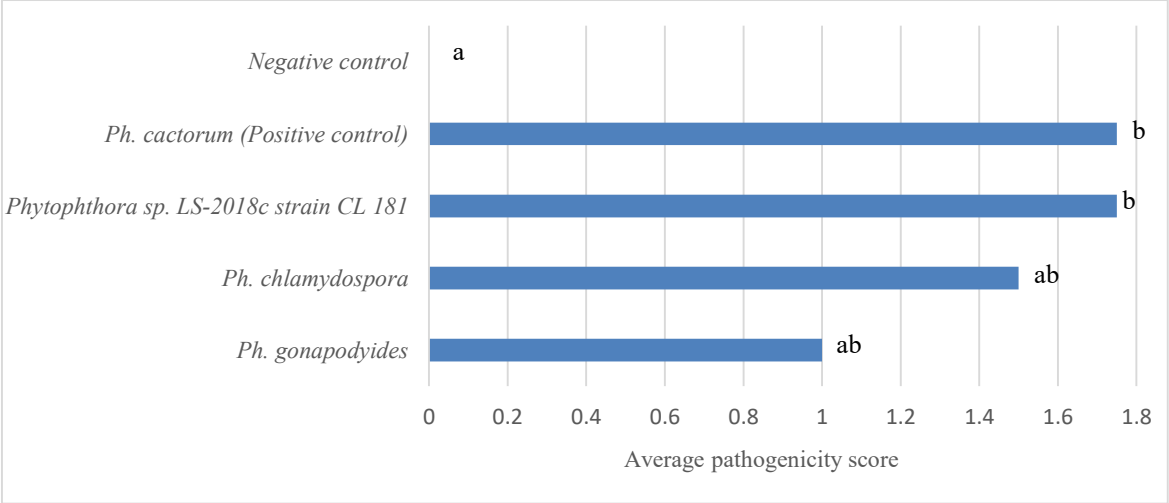

Figure 4.5: Average pathogenicity score on lupin seedling after inoculation with four *Phytophthora* spp. isolates (*Phytophthora* sp. LS-2018c strain CL 181, *Ph. chlamydospora*, *Ph. gonapodyides* and *Ph. cactorum* (positive control)) compared with the negative control. Bars with different letters are significantly different at P=0.05.

No lesions developed on any of the lupin seedlings after 7 days incubation in sterile water (non-chlorinated tap water and deionised water (Section 4.2.1) inoculated with mycelium colonised agar plugs of *Phytophthora* sp. LS-2018c strain CL 181, *Ph. chlamydospora* and *Ph. gonapodyides* or the positive control, *Ph. cactorum* indicating that the *Phytophthora* spp. isolates had not caused any infection. No *Phytophthora* colonies were obtained from re-isolations from the lupin roots on *Phytophthora* selective media (Figure 4.6).

Figure 4.6: Lupin seedlings with no lesions observed after seven days incubation in soil extract solution inoculated with a *Phytophthora* mycelium colonised agar disc.

No lesions developed on any of the lupin seedlings after 7 days incubation in sterile soil extract solution (Section 4.2.2) inoculated with mycelium colonised agar plugs of *Phytophthora* sp. LS-2018c strain CL 181*, Ph. chlamydospora* and *Ph. gonapodyides* or the positive control (*Ph. cactorum)* again indicating that the *Phytophthora* spp. isolates had not cause any infections. No *Phytophthora* colonies were obtained from re-isolations from the lupin roots on *Phytophthora* selective media.

The pathogenicity test using agar plugs colonised with the *Phytophthora* spp. isolates to inoculated lupin seedlings grown in sterile vermiculite was not successful (Section 4.2.4). Wilting and discoloration of the lupin seedling roots were observed however, no *Phytophthora* spp. colonies were isolated on the isolation plates from the infected material. Similar symptoms were also observed on the negative control, indicating that any visual symptoms occurring on the lupins were not caused by the *Phytophthora* spp. isolates used for inoculation but likely due to contamination by another pathogenic microorganism, however no colony growth was observed during re-isolation on P_5CMA containing antibiotics (Figure 4.7).

Figure 4.7: Lesions (red arrow) observed on lupin seedlings grown in vermiculite inoculated with *Phytophthora* colonised agar plugs or uninoculated control, (A) visible lesion and discoloration of lupin seedling inoculated with *Phytophthora* sp. LS-2018c strain CL 181, and (B) lesion and softening of tissue on negative control.

4.4 Discussion

This chapter tested four methods to identify a rapid assay that could be used to screen large numbers of *Phytophthora* species and strains isolated from waterways for pathogenicity using lupin seedlings. Of the four methods tested only one was successful in enabling the pathogenicity to be evaluate whereby symptoms/lesions were observed and *Phytophthora* colonies were recovered from the inoculated seedlings, thereby confirming Koch's postulates. This method involved inoculating *Phytophthora* isolates using agar plugs on lupin seedlings grown on sterile paper towels and was a rapid test for determining the pathogenicity of the *Phytophthora* isolates recovered from the waterways with results observed 4 days post inoculation. In contrast, the other three methods (Sections 4.2.1, 4.2.2 and 4.2.4) did not work. Pathogenicity tests on seedling using direct inoculation of roots with mycelial colonised agar plugs has been previously used to test the pathogenicity of *Ph. parasitica* on tomato and pepper (Boix-Ruiz *et al.*, 2017). The current study also looked at evaluating the pathogenicity of *Phytophthora* spp. isolates on lupin seedlings by submerging *Phytophthora* infected agar plugs in sterile water (tap water and distilled water) and sterile soil extract solution, which are used commonly in inducing *Phytophthora* zoospore production (Drenth & Sendall, 2001; Jeffers, 2015a; Jeffers, 2006). According to Jeffers (2006), completely submerging the agar plugs in liquid inhibits sporangia production, and hence zoospore inoculum production, which may explain why no lesions were obtained on the lupin radicles in this study. This experiment could have been further refined by determining the presence of zoospores in the sterile water and soil extract solution. Pathogenicity testing of the *Phytophthora* spp. isolates by inoculating lupin seedlings grown in vermiculite was also not successful which indicates that this experimental set up did not provide an ideal condition for *Phytophthora* to sporulate and cause infection. Vermiculite was used as it has been commonly used for production of *Phytophthora* inoculum (Ivors, 2015b). However, oat seed and V8 broth are also used to amend the vermiculite although this mixture only promotes mycelium growth with longer time (3-5 weeks) required for colonization. This could explain why no *Phytophthora* infection was seen on the lupins even after 2-weeks post inoculation in vermiculite, as the mycelium may not have reached the lupin roots. Previous pot experiments have inoculated seedlings by incubating roots in a *Phytophthora* zoospore suspension prior to planting in pots, with disease symptoms visible on the lupins 5 to 6 days post inoculation (Beligala, 2016; Widmer *et al.*, 2012). In pot trials, Parke and Lewis (2007) inoculated nursery grown rhododendron plants with *Ph. ramorum* inoculum (V8 broth vermiculite culture and chopped infected rhododendron leaves) by stimulating *Phytophthora* sporulation by flooding the potting media for 12 hrs., however, in the current experiment only vermiculite was used which drained faster and most likely had a short flooding period that did not stimulate sporulation and infection. Therefore, from the results of the study, pathogenicity screening of *Phytophthora* spp. isolates using colonised agar plugs to inoculate lupin seedlings grown on sterile paper towels can be used for quick identification of pathogenic isolates from waterways. The pathogenicity of isolates identified using this initial lupin pathogenicity screen should however then be

determined on other agricultural crops and native and exotic trees and shrubs of economic or conservational significance in further pot experiments to evaluate the potential risk of these species.

A large number of *Phytophthora* hybrids were recovered from the waterways in this study (Chapter 2 and 3), however their pathogenicity is unclear. Although *Phytophthora* hybrids have been frequently recovered from waterways (Burgess, 2015; Hüberli *et al.*, 2013; Nagel *et al.*, 2013) their pathogenicity has not been determined in these studies. For example, although *Phytophthora×stagnum* nothosp. nov. a hybrid of *Ph. chlamydospora* and an unknown species genetically close to *Ph. mississippiae*, was frequently recovered from several irrigation reservoirs in ornamental plant nurseries in Virginia its pathogenicity on nursery plants has not been determined (Yang *et al.*, 2014). For the hybrids recovered in this study, further work is needed to determine their pathogenicity and the pathogenicity assay developed could be used to determine their potential pathogenicity.

Phytophthora sp. LS-2018c strain CL 181*, Ph. chlamydospora* and *Ph. gonapodyides* were found to be equally pathogenic on lupin seedlings and had similar pathogenicity to a known pathogen, *Ph. cactorum* (Hantula *et al.*, 2000) on lupin. *Phytophthora* sp. LS-2018c strain CL 181 is a new species isolated in Chile, with this study being the first to detect this species in New Zealand and its pathogenicity yet to be explored. *Phytophthora* sp. LS-2018c strain CL 181 was found to be pathogenic on *Quercus suber* (Cork Oak) found in Valdivian rainforest (Jung *et al.*, 2018a). Since *Q. suber,* is grown as an ornamental tree throughout New Zealand, including Canterbury, its potential as a host of this species needs to be investigated (Thorpe, 2017). Further research on possible hosts and the pathogenicity of this species is required to understand the potential risk in New Zealand. *Phytophthora chlamydospora* was commonly recovered as a hybrid in Canterbury waterways and has been long known to be associated with exotic and native trees in New Zealand (Section 2.4.4). Commonly isolated from soils of native forests, irrigation systems and waterways, *Ph. chlamydospora* has been found to be an opportunistic and sometimes aggressive pathogen of trees (Aghighi *et al.*, 2016). Due to its broad host range and its opportunistic nature, pathogenicity of *Ph. chlamydospora* and its hybrids, should be evaluated on other plants especially native and exotic trees and shrubs, incorporating factors causing stress that are likely to make the plants more susceptible. *Phytophthora gonapodyides* included in this study is an established species with known pathogenicity to trees and fruits trees in New Zealand (Manaaki Whenua Landcare Research, 2019a; Stewart & McCarrison, 1991). Similar to *Ph. chlamydospora, Ph. gonapodyides* is also an opportunistic and sometimes aggressive pathogen of trees (Aghighi *et al.*, 2016). *Phytophthora gonapodyides* has a wide host range (Section 2.4.7) and has been associated with agricultural and horticultural crops (Jung *et al.*, 2011). Climatic triggers such as high precipitation and mild winter temperatures in Sweden was reported to provide favourable conditions for *Ph. gonapodyides* to spread with old beech stands being more susceptible favouring stem canker disease (Cleary *et al.*, 2016). *Phytophthora gonapodyides* is likely to be wide spread in New Zealand and further research on the potential pathogenicity of *Ph. gonapodyides* under climate change conditions is needed to identify the possible implications of this pathogen on New Zealand's native ecosystem.

The current study screened the pathogenicity of three clade 6 *Phytophthora* spp., however, further pathogenicity screening is required to determine the pathogenicity and potential risk of the other species isolated from the Canterbury waterways. Further, in this study only one isolate of each species was used in the pathogenicity assays, and whether the relative pathogenicity of isolates within a species varies is unknown and warrants further investigation.

4.5 Conclusion

Phytophthora sp. LS-2018c strain CL 181, *Ph. chlamydospora* and *Ph. gonapodyides* were found to be pathogenic on lupin seedlings. Pathogenicity tests using direct inoculation of lupin roots with *Phytophthora* colonised agar plugs was identified as the preferred method for rapid pathogenicity screening of *Phytophthora* spp. isolated from waterways. This method is quick and easy as it only takes 4 days post inoculation to obtain the pathogenicity test results. Isolates of different *Phytophthora* spp. recovered from Canterbury waterways should be screened for pathogenicity on lupin seedlings and the pathogenic isolates should be further tested for pathogenicity on agricultural crops and native and exotic trees and shrubs of economic or conservational significance to identify the risk these isolates pose in New Zealand.

Chapter 5: Concluding discussion

This was the first study to identify the *Phytophthora* spp. present in waterways in Canterbury, New Zealand. The first aim of this study was to identify the best *Phytophthora* recovery method and best leaf bait (Chapter 2) which was used for a more extensive study to identify the diversity of *Phytophthora* spp. in Canterbury waterways (Chapter 3). *Phytophthora* spp. diversity study was carried out in six Canterbury waterways with 25 sites in autumn (May 2018). Additionally, four methods were tested to identify a pathogenicity assay that could be used to rapidly screen and identify pathogenic *Phytophthora* spp. isolates recovered from waterways (Chapter 4). A rapid pathogenicity screening assay for testing *Phytophthora* spp. recovered from waterways will be useful to identify pathogenic isolates that can then be assessed in further pathogenicity tests using native and exotic trees and shrubs and agricultural crops. This Chapter looks at the overall findings of this study and identifies recommendations for improvements for future *Phytophthora* diversity study.

Identification of the best method for isolating *Phytophthora* species from waterways in Canterbury

The study identified water baiting, either laboratory baiting or river baiting, as being suitable methods to study *Phytophthora* spp. diversity in waterways. The filtration method was not effective due to the high number of *Pythium* colonies recovered which contaminated the *Phytophthora* colonies. Currently, the river baiting (*in situ*) method is the most widely used method for *Phytophthora* spp. recovery from ponds, rivers, lakes and streams (APHIS, 2014) as it allows the leaf baits to have longer exposure to the *Phytophthora* spp. inoculum in the waterways. Laboratory baiting is popular for *Phytophthora* spp. recovery in intermittent streams in forests and water sources in nurseries, and is effectively used for *Ph. ramorum* surveillance in USA (APHIS, 2014; Ivors, 2018; Parke & Rizzo, 2011) and the results of this study indicate that it could be used to survey New Zealand waterways to determine *Phytophthora* spp. diversity. Seven leaf baits (*R. arboreum*, *Pt. undulatum, B. attenuata, Ca. japonica*, *Pt. eugenioides, Pinus radiata* and *Ce. deodara*) were evaluated for their effectiveness at recovering *Phytophthora* spp. from waterways. Regardless of the baiting method used in Chapter 2, *R. arboreum* was identified as the preferable bait for recovering larger numbers of *Phytophthora* isolates compared with the other six leaf baits, while there was no difference in the number of species recovered. When the leaf baits were evaluated using a larger sample size (25 sites; Chapter 3) *R. arboreum* was found to recover the highest number of *Phytophthora* isolates and recovered all the *Phytophthora* spp. isolated in the study. *Rhododendron arboreum* was also found to be more tolerant to the fluctuating water pH than *Pi. radiata* and *Ce. deodara,* which resulted in a more consistent *Phytophthora* recovery irrespective of water pH, within the range found in the study. As identified in this study future *Phytophthora* spp. diversity studies should focus on using laboratory baiting and river baiting method with *R. arboreum* leaf bait.

Identification of *Phytophthora* species diversity in Canterbury waterways

In the *Phytophthora* spp. diversity study (autumn baiting), *Ph. lacustris* was the most commonly isolated species recovered from 19 of the 25 sites. This was followed by *Ph. gonapodyides* recovered from 14 sites, *Ph. chlamydospora* x *Ph. amnicola* hybrid recovered from 15 sites and *Ph. thermophila x amnicola* hybrid recovered from eight sites. Low numbers of *Ph. chlamydospora* x *Ph. thermophila* hybrid (three isolates; from three sites), *Ph. chlamydospora* (three isolates; from three sites), *Ph. amnicola* (three isolates; three sites) and *Ph. bilorbang* (one isolate) was also recovered. *Phytophthora* sp. LS-2018c strain CL18, *Ph. cryptogea* and *Ph. cactorum* was recovered from the Halswell River in summer baiting (February 2018). This is a similar diversity to what has been reported from other waterways baiting surveys (Hüberli *et al.*, 2013; Randall, 2011; Reeser *et al.*, 2011).

Prior to this study, only a few *Phytophthora* spp. were reported in the Canterbury region, all of which were isolated from diseased plant and soil (Manaaki Whenua Landcare Research, 2019d). This study reports the first recovery of some species or hybrids in the Canterbury region including *Ph. thermophila x amnicola* hybrid, *Ph. chlamydospora* x *Ph. thermophila* hybrid, *Ph. amnicola x Ph. chlamydospora* hybrid, *Ph. chlamydospora, Ph. bilorbang, Ph. lacustris,* and *Phytophthora* sp. LS-2018c strain CL181. *Phytophthora gonapodyides* and *Ph. cryptogea* were also recovered however, these species have been previously reported to be widespread throughout New Zealand (Manaaki Whenua Landcare Research, 2019a). *Phytophthora cactorum* has mostly been found in association with orchards in New Zealand, however this study reports the first isolation from a New Zealand waterway. However, this study reports for the first time the presence of *Phytophthora* sp. LS-2018c strain CL181 and *Ph. thermophila* in New Zealand, identifying these as being new species in New Zealand which has been reported to the Ministry of Primary Industries (MPI).

Pathogenicity of *Phytophthora* spp. recovered from waterways

This study aimed to determine the pathogenicity of three commonly recovered *Phytophthora* spp. from Canterbury waterways; *Phytophthora* sp. LS-2018c strain CL 181, *Ph. chlamydospora* and *Ph. gonapodyides* (Chapters 2 and 3). The evaluation of four methods to identify a rapid pathogenicity screening assay that could be used for future screening of *Phytophthora* spp. isolates recovered from waterways, was undertaken. Testing the pathogenicity of *Phytophthora* spp. isolates using agar plugs on lupin seedlings grown on sterile paper towel was identified as a good rapid test to determine the pathogenicity of *Phytophthora* isolates recovered from waterways, with results observed 4 days post inoculation. Using this assay all an isolate of each of the three *Phytophthora* species (*Phytophthora* sp. LS-2018c strain CL 181, *Ph. chlamydospora* and *Ph. gonapodyides)* were found to be pathogenic on lupin seedlings. *Phytophthora* isolates that are shown to be pathogenic in this pathogenicity screening assay should be included in further pathogenicity tests using crop plants, fruit trees, native and exotic tree/shrub species to evaluate the risks these species pose to New Zealand's agriculture and the native ecosystem.

Evaluation of the *Phytophthora* recovery method and recommendations for future research

Phytophthora recovery for this research has depended on isolating *Phytophthora* isolates that have caused infections on leaf baits suspended into the water sample (laboratory baiting) or in rivers (*in situ*). The *Phytophthora* species recovered in this study are therefore ones that were able to cause lesions on the leaf baits. Other studies of *Phytophthora* spp. diversity from waterways have used similar methods to the current study, whereby *Phytophthora* isolates were recovered from lesions on leaf baits plated on *Phytophthora* media with identification of the isolates through morphology and DNA identification of the *Phytophthora* cultures (Burgess *et al.*, 2009; Hüberli *et al.*, 2013; Reeser *et al.*, 2011; Stamler *et al.*, 2016a). However, the question remains as to whether there are other *Phytophthora* spp. present in the waterways that could not cause infection of the leaf baits, or the lesions on leaf baits took a longer time to develop than the 7 days incubation period allowed, or the *Phytophthora* sp. infected the leaf bait but was asymptomatic and would not be isolated in this study. According to Redondo (2018) the study of *Phytophthora* spp. communities by standard methods of isolation of *Phytophthora* cultures from substrate (plant material, soil and water) is time consuming and can be biased. For example, *Ph. cinnamomi* is difficult to isolate from waterways and this gives a false negative through baiting. Burgess *et al.* (2016) also supports historical bias in detecting *Phytophthora* spp. diversity as traditional isolation methods have variable effectiveness in detecting different *Phytophthora* spp. A new technique for *Phytophthora* spp. detection from environmental samples has been developed whereby DNA is extracted from the environmental samples (plant material, soil and water) followed by metabarcoding using high-throughput sequencing (HTS) (Burgess *et al.*, 2016; Redondo, 2018). This technique allows rapid characterization of microbial communities without relying on the ability to isolate and culture the target organisms from the samples on culture media (Català *et al.*, 2017). This technique is reported to be a more efficient method for *Phytophthora* spp. community studies as it detects three time more *Phytophthora* spp. than traditional methods (Català *et al.*, 2017; Redondo, 2018). However, HTS has some drawbacks, as *Phytophthora* spp. recovery depends on the substrate the DNA is isolated from. Plant baits incubated in waterways tend to isolate more *Phytophthora* spp. than filtration of water sample, as the baits tend to have a longer exposure to the species present in waterways whereas, DNA obtained from water filtration only targets species present in the often small volume of water used (Redondo, 2018). To confirm the results of metabarcoding and rule out the possibility of sample contamination additional methods such as real-time PCR are also required to assess the presence of *Phytophthora* spp. in the environmental samples (Català *et al.*, 2017). However, although metabarcoding using HTS method is an effective method for identifying the *Phythophthora* spp. communities in environmental samples, this in itself will not result in a culture collection of *Phytophthora* spp. isolates that can be use in other studies such as determining the pathogenicity of the isolates/species recovered. This study used a baiting method for *Phytophthora* spp. isolate recovery because another aim of the study was to develop an assay which could be used to evaluate the pathogenicity of selected *Phytophthora* spp. isolates. For future studies of the *Phytophthora* spp. diversity from waterways it is recommended that HTS method using

DNA extracted from both plant baits and water filtration since it is more efficient in determining the *Phytophthora* spp. diversity. Representative isolates should also be isolated from leaf baits into culture for pathogenicity or other studies which required live cultures.

Another potential issue is that using HTS method to determine *Phytophthora* spp. communities is relatively expensive, whereas morphological grouping of isolates followed by DNA identification of representative isolates is still a good and relatively inexpensive option for *Phytophthora* studies. Similar to other *Phytophthora* studies (Burgess *et al.*, 2016; Hüberli *et al.*, 2013; Redondo, 2018; Reeser *et al.*, 2011; Sutton *et al.*, 2009) the current study used sequencing of the ITS gene region to identify the isolates to species level. However, the ITS sequence results were found to not provide accurate identification of the isolates, and in many cases was not able to distinguish between closely related species and identify potential hybrids. This has been reported by other researchers (Burgess, 2015; Hüberli *et al.*, 2013; Nagel *et al.*, 2013) and based on the literature on *Phytophthora* species identification (Hwang *et al.*, 2008; Kroon *et al.*, 2004; Martin & Tooley, 2003) and advice from Dr Stan Bellgard and Dr Chantal Probst (Maanaki Whenua-Landcare Research, Auckland) sequencing of the *coxI* gene region was used to provide a more accurate species identification. Further, using this gene region, a large number of *Phytophthora* hybrid isolates were also identified. Therefore, for future work identification of the *Phytophthora* isolates recovered from waterways using the sequence of the *coxI* gene region is recommended.

In this study, the temperature range of the water in the waterways at the sites sampled for *Phytophthora* recovery was 4°C to12°C, however, isolation of the *Phytophthora* isolates in the laboratory was carried out at 20°C. From the water samples taken from the river sites which had an *in-situ* water temperature of 4°C a low number of *Phytophthora* isolates and species was recovered, with only one isolate/species recovered from Selwyn site 6 (*Ph. lacustris*) and Ashburton site 3 (*Ph. chlamydospora x Ph. amnicola* hybrid), while no *Phytophthora* isolates were recovered from Ashburton site 4. The question remains whether there were other *Phytophthora* spp. adapted to colder water environments that could not be recovered at an incubation temperature of 20°C. The optimum growth temperature is known to vary amongst different *Phytophthora* spp. (Gallegly & Hong, 2008b) with higher laboratory incubation temperature for the species isolated from colder environments likely to create an unfavourable growing condition for some *Phytophthora* spp. The use of 20°C for incubation and isolation of the *Phytophthora* spp. in this study is similar to that used in other previous studies on *Phytophthora* recovery from waterways (Burgess *et al.*, 2016; Hüberli *et al.*, 2013; Redondo, 2018; Reeser *et al.*, 2011; Sutton *et al.*, 2009). The impact of incubating temperature on the recovery of different *Phytophthora* spp. from waterways, or other environmental samples, is yet to be investigated. To investigate the effect of incubation temperature on the recovery of *Phytophthora* spp., the HTS method should be used together with the traditional baiting and culturing method with incubation at different temperatures for comparison of the species recovered from sites with varying water temperatures.

Evaluation of the sampling sites and recommendations for future research

The effect of land use type on *Phytophthora* spp. diversity in waterways is supported by the findings of Redondo *et al.* (2018) where agricultural areas had higher *Phytophthora* spp. diversity than in native forests, with the distribution of pathogenic *Phytophthora* spp. being linked to plant diversity. In this study, two large rivers (Ashburton River and Selwyn River) were sampled from the foothills out towards the sea with an assumption that the land use and abiotic water parameters would vary at different sites. However, the assessment of the impact of land use on *Phytophthora* spp. diversity in this study was likely affected by the relatively high number of braided river sites (Ashburton River sites 1,2 ,5 and 6, Selwyn River sites 2, 7 and 8) and the similarity in the riparian vegetation in a number of the sampling sites which reduced the difference in the sampling sites. The presence of large areas of bush and bare rocky ground on the braided river banks presented harsh conditions for zoospore survival preventing the *Phytophthora* spp. present in the surrounding land area from entering the waterways. Therefore, the species recovered along the braided river may not be a true representation of the *Phytophthora* spp. present in the surrounding land area. Further, similar riparian plants (willow, poplars and other bushes) were found on the river banks for many of the other sites sampled for the Selwyn River (sites 1, 3, 4, 5 and 6) which may have affected the *Phytophthora* spp. diversity. Additionally, previous study has linked the difference in the water chemistry (pH, conductivity, total organic carbon, and total nitrogen) between environments to the different aquatic *Phytophthora* spp. communities recovered from river sites located in cities, agricultural fields and in forests (Redondo, 2018). However, sites sampled in this study consisted of mainly waterways running through agricultural land associated with riparian plants consisting of exotic and native trees and shrubs, and conservation areas (native trees/shrubs) which had little variation in the abiotic water parameters (pH, temperature, salinity, nitrate and ammonium) between the sampling sites resulting in the lack of a significant relationship between *Phytophthora* spp. diversity and abiotic water parameters. In contrast the study of Redondo (2018) found differences in the water chemistry between waterways in cities, agricultural fields and forest areas. As the *Phytophthora* spp. diversity in waterways has been linked to land use, plant diversity, and the water chemistry of the sites, site selection for baiting becomes an important process in *Phytophthora* spp. diversity study. Sites that directly pass through a land use is more suitable for sampling than rivers sites which are braided. Further, a greater number of *Phytophthora* spp. (five *Phytophthora* spp.) were recovered from Kaituna Valley site 3 which was a small stream (Okana stream) than the river sites in the Kaituna Valley (site 2; four *Phytophthora* spp. and site 3; two *Phytophthora* spp.) as there is a possibility of *Phytophthora* inoculum becoming diluted once they enter larger waterways. Future *Phytophthora* baiting should be done in streams that pass directly through the land use as this will give a better representation of the *Phytophthora* spp. present in an area. Since land use affects the quantity and quality of water nutrients which influences water aquatic *Phytophthora* communities (Redondo *et al.*, 2018) future water baiting should include more diverse sites in terms of land use, especially urban areas which are likely to have different water chemistry with abiotic factors assessed over a larger number of sites. For example, two

rivers that flow through Christchurch, the Heathcote River flowing from the Port Hills through more industrial areas to the sea and the Avon River which flows through the Botanic Gardens, the central city and through the earthquake red zone out to sea should be sampled to determine whether the different land use affect the *Phytophthora* spp. communities recovered.

Lakes should also be included in future *Phytophthora* spp. diversity studies as lakes are believed to be reservoirs of aquatic microbial communities where the rivers and streams empty into. Lake Ellesmere is fed by numerous streams and rivers from Banks Peninsula and the Canterbury plains including the Halswell River, Kaituna River, Prices Valley River, and the Selwyn River and would be a good site to sample for *Phytophthora* spp. Lake Ellesmere is considered to be a sink of nutrients contributed by the agriculturally based catchment (Hughey & Taylor, 2009) and is fed by 40 rivers and streams that flow adjacent to farmlands and is dominated by nitrate- enriched ground water fed streams (Hughey & Taylor, 2009). In this study, Lake Ellesmere was not included as its considered to have brackish, saline water due to it often being open to the sea through the creation of artificial channels. Further, according to Spigel (2009) salt water intrusion causes high salinity near the lake outlet however, salinity levels were found to be lower where the lake is fed by the freshwater from rivers and streams with the salinity levels in different sites in the lake ranging from 2 ppt to 12 ppt. As salinity has been found to have a negative correlation with the number of *Phytophthora* spp. in waterways (Redondo *et al.*, 2018), areas of lake where the freshwater enters the lake would be more suitable for *Phytophthora* baiting. Due to the contribution of nitrate- enriched ground fed streams, Lake Ellesmere is expected to have a higher nitrogen level than the waterways baited in the current study. According to the findings of Redondo *et al.* (2018), total nitrogen had a positive correlation with the number of *Phytophthora* spp. and likely to support a higher *Phytophthora* spp. diversity in the lake. Lake Ellesmere has not been included in previous *Phytophthora* spp. diversity studies and baiting different sites in the lake for *Phytophthora* spp. is important as it is likely to give a better representation of the species present in the Canterbury region. Apart from this, several other lakes exists in Canterbury region such as Lake Clearwater, Lake Camp, Lake Heron, Lake Emma, Lake Emily, Lake Denny, Lake Roundabout, Spider Lakes, and the Maori Lakes which should be included in future *Phytophthora* diversity studies.

In conclusion, this is the first study to recover *Phytophthora* spp. from Canterbury waterways, from which five species and three hybrid species were identified. Of these, this was the first recovery of two species (*Phytophthora* sp. LS-2018c strain CL 181 and *Ph. thermophila* hybrids) from New Zealand. The indication that isolates of three of the species, *Ph. chlamydospora*, *Ph. gonapodyides* and *Phytophthora* sp. LS-2018c strain CL 181, were potential pathogens, and the recovery of *Ph. cactorum* which is a recognised pathogen of a range of plant hosts, is of potential importance to any grower that sources water for irrigation from these waterways. Further work is required to determine the risk of these *Phytophthora* species to agricultural crops, and also native ecosystems.

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Appendix A.1: Supplementary material for chapter 1

A.1.1

Table A1: *Phytophthora* spp. present in New Zealand, source Scott and Williams (2014), Lewis. K (2018) and Manaaki Whenua Landcare Research (2019)

No.	***Phytophthora* spp. present in New Zealand**	**Reference**
1	*Phytophthora cinnamomi*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
2	*Phytophthora kernoviae*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
3	*Phytophthora multivora*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
4	*Phytophthora cactorum*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
5	*Phytophthora infestans*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
6	*Phytophthora nicotianae*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
7	*Phytophthora citricola*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
8	*Phytophthora citrophthora*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
9	*Phytophthora meadii*	Scott and Williams (2014)
10	*Phytophthora multivesiculata*	Scott and Williams (2014)
11	*Phytophthora plurivora*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
12	*Phytophthora pluvialis*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
13	*Phytophthora agathidicida (Phytophthora* taxon agathis *(PTA))*	Scott and Williams (2014)
14	*Phytophthora asparagi*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
15	*Phytophthora gonapodyides*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
16	*Phytophthora megasperma*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
17	*Phytophthora chlamydospora (Phytophthora* taxon PgChlamydo*)*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
18	*Phytophthora brassicae*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
19	*Phytophthora cryptogea*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
20	*Phytophthora drechsleri*	Scott and Williams (2014)
21	*Phytophthora erythroseptica*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
22	*Phytophthora hibernalis*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)

Table A1 continued

No.	*Phytophthora* spp. present in New Zealand	Reference
23	*Phytophthora porri*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
24	*Phytophthora primulae*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
25	*Phytophthora syringae*	Scott and Williams (2014)
26	*Phytophthora captiosa*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
27	*Phytophthora fallax*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
28	*Phytophthora cambivora*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
29	*Phytophthora fragariae*	Scott and Williams (2014) Manaaki Whenua Landcare Research (2019)
30	*Phytophthora pini*	Lewis. K (2018)
31	*Phytophthora gregata*	Lewis. K (2018)
32	*Phytophthora aleatoria*	Manaaki Whenua Landcare Research (2019)
33	*Phytophthora amnicola*	Manaaki Whenua Landcare Research (2019)
34	*Ph. amnicola x Ph. chlamydospora* hybrid	Manaaki Whenua Landcare Research (2019)
35	*Phytophthora bilorbang*	Manaaki Whenua Landcare Research (2019)
36	*Phytophthora boehmeriae*	Manaaki Whenua Landcare Research (2019)
37	*Phytophthora europaea*	Manaaki Whenua Landcare Research (2019)
38	*Phytophthora inflata*	Manaaki Whenua Landcare Research (2019)
39	*Phytophthora inundata*	Manaaki Whenua Landcare Research (2019)
40	*Phytophthora lacustris*	Manaaki Whenua Landcare Research (2019)
41	*Phytophthora medicaginis*	Manaaki Whenua Landcare Research (2019)
42	*Phytophthora niederhauserii*	Manaaki Whenua Landcare Research (2019)
43	*Phytophthora pseudocryptogea*	Manaaki Whenua Landcare Research (2019)

Appendix A.2: Supplementary material for chapter 2

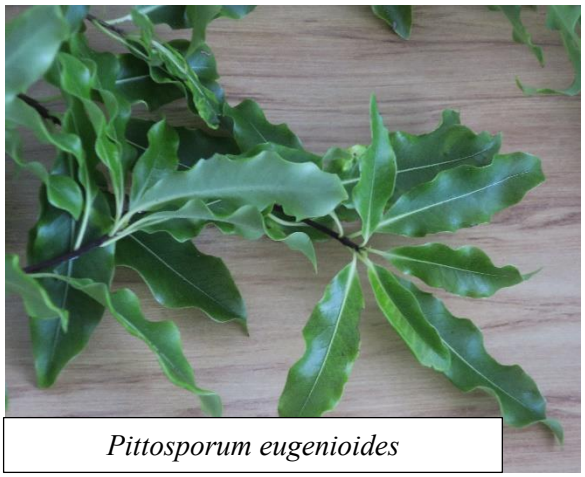
A.2.1 Seven leaf baits.

Rhododendron arboreum

Pinus radiata

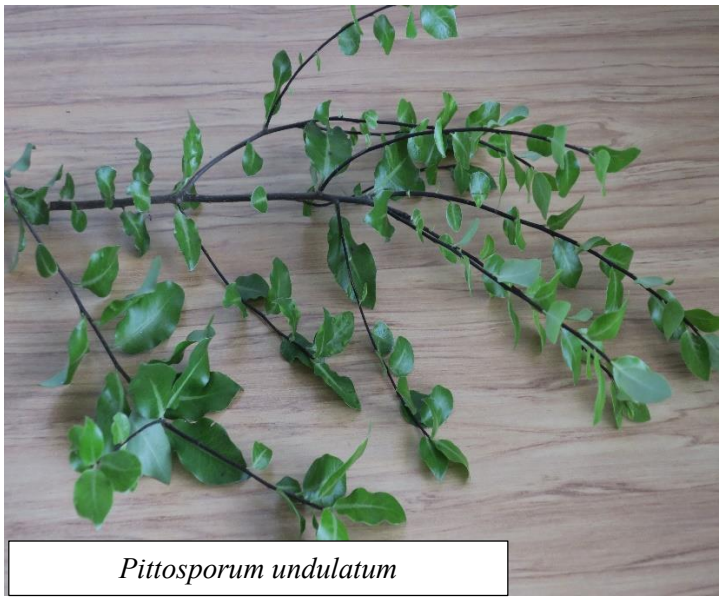
Cedrus deodara

Banksia attenuata



Pittosporum eugenioides

A.2.1 continued.



Pittosporum undulatum

Camellia japonica

A.2.1 Agar Recipes

Phytophthora selective media

17 g CMA in 1 L of distilled water autoclaved and cooled to 50^{o}C.

Amended with 5 µg/mL pimaricin, 250 µg/mL ampicillin, 10 µg/mL rifampicin and 100 µg/mL pentachloronitrobenzene either without (P_5 ARP CMA) or with 50 µg/mL hymexazol (P_5 ARPH CMA) prior to pouring the agar.

20% V8A

200 mL V8 juice, 800 mL distilled water, 2 g $CaCO_3$, 17 g Davis standard agar.

PDA

39 g PDA (difco^{TM}) 1 L distilled water.

1 % Soil Extract solution

Non-sterile soil extract solution (NSSES)- 10 g of soil, 1 L distilled water, stirred for 4 hrs and filtered using 2 layers of Whatman #1 filter paper.

Sterile soil extract solution (SSES)- NSSES solution autoclaved.

TAE buffer

50X TAE- 242 g Tris-base, 57.1 mL Glacial acetic acid, 100 mL 0.5M EDTA (pH), make up to 1 L with distilled water.

Buffer for Agarose gel: 40 mL of 50X TAE in 2 L of distilled water.

A.2.3 GPS coordinates for the 6 sites surveyed for *Phytophthora.*

Sampling date	River	site	GPS Latitude	GPS Longitude
13/03/2018	Halswell	1	-43.661992019042373	172.54232998006046
13/03/2018	Halswell	2	-43.656850978732109	172.51537696458399
2/02/2018	Liffey	1	-43.638800969347358	172.48596796765924
2/02/2018	Liffey	2	-43.641865979880095	172.48701503500342
2/02/2018	Liffey	3	-43.644907018169761	172.48950404115021
2/02/2018	Liffey	4	-43.647533990442753	172.4908690340817

A.2.4 Master mix for PCR amplification

Table A.1: Master mix for 18ph2F and 28ph2R (ITS) amplification

Component	Final concentration	Final volume used
DNA	10 ng/ µL	2 µL
Dream *Taq* (x2)	1 unit	10 µL
18ph2F 10 µM stock	0.2 µM	0.4 µL
28ph2R 10 µM stock	0.2 µM	0.4 µL
Deionised water		7.2 µl

Table A.2: Master mix for FM84 and FM 77 (*coxI)* amplification

Component	Final concentration	Final volume used
DNA	10 ng/ µL	2 µL
Dream Taq (x2)	1 unit	10 µL
FM84 10 µM stock	0.2 µM	0.4 µL
FM 77 10 µM stock	0.2 µM	0.4 µL
Deionised water		7.2 µL

A.2.5 DNA sequence of *Phytophthora* isolates: ITS and *coxI.*

ITS sequences for *Phytophthora* isolates obtained from Halswell River

T5

GCCCGTCGCACCTACCGATTGAATGACTCGGTGAAAAATTGGGACCGTGAGTCCGCTTGCTTCATTGCGAGTGG
ATTGATGGGAACTTTTTTAAACCTCGCCATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCT
GCGGAAGGATCATTACCACACCTAAAAAAACTTTCCACGTGAACACGTATCAACCCTTTTAGTTGGGGGTCTTG
TACCCTATCATGGCGAATGTTTGGACTTCGGTCTGGGCGAGTAGCCTTTTGTTTTAAACCCATTTCACAATACTG
ATTATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCAC
ATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAA
CGCATATTGCACTTCCGGGTTAGTCCTGGGAGTATGCCTGTATCAGTGTCCGTACATCAAACTTGGCTTTCTTCC
TTCCGTGTAGTCGGTGGAGGATGTGCCAGATGTGAAGTGTCTTGCGTGTTGTCCTTCGGGTCGGCTGCGAGTCC
TTTTAAATGTACTGAACTGTACTTCTCTTTGCTCAAAAAGCGTGGTGTTGCTGGTTGTGGAGGCTGCTTGCGTGG
CCAGTCGGCGACCGGTTTGTCTGCTGCGGCGTTTAATGGAGGAGTGTTCGATTCGCGGTATGGTTGGCTTCGGC
TGAACAGGCGCTTATTGTATGCTTTTCCTGCTGTGGCGTGATGGGCTGGTGAACCGTAGCTGTGTGTGGCTTGG
CTTTTGAATCGGCTTT

T6

ACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGACTCGGTGAAAAATTGGGACCGTGA
GTCCGCTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCGCCATTTAGAGGAAGGTGAAGTCGT
AACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAAAAAACTTTCCACGTGAACCGTAT
CAACCCTTTTAGTTGGGGGTCTTGTACCCTATCATGGCGAATGTTTGGACTTCGGTCTGGGCGAGTAGCCTTTTG
TTTTAAACCCATTTCACAATACTGATTATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTC
AGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGAT
TCAGTGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTAGTCCTGGGAGTATGCCTGTATCAGTGTC
CGTACATCAAACTTGGCTTTCTTCCTTCCGTGTAGTCGGTGGAGGATGTGCCAGATGTGAAGTGTCTTGCGTGT
TGTCCTTCGGGTCGGCTGCGAGTCCTTTTAAATGTACTGAACTGTACTTCTCTTTGCTCAAAAAGCGTGGTGTTG
CTGGTTGTGGAGGCTGCTTGCGTGGCCAGTCGGCGACCGGTTTGTCTGCTGCGGCGTTTAATGGAGGAGTGTTC
GATTCGCGGTATGGTTGGCTTCGGCTGAACAGGCGCTTATTGTATGCTTTTCCTGCTGTGGCGTGATGGGCTGG
TGAACCGTAGCTGTGTGTGGCTTGGCTTTTGAATCGGCTTTGCTGTTGCGAAGTAGAGTGGCGGCTTCGGCTGT
CGAGGGTCGATCCATTTTGGGAACTTTGTGTGCACTTCGGTGCGCATCTCAATTGGACCTGATATCAGGCAAGA
TTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACGGCGAG
TGAAGCGGGAAGAGCTCAAGCTT

T8

CACCGCCCGTCGCACCTACCGATTGAATGACTCGGTGAAAAATTGGGACCGTGAGTCCGTTTGCTTCATTGCGA
GTGGATTGATGGGAACTTTTTTAAACCTCGCCATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTG
AACCTGCGGAAGGATCATTACCACACCTAAAAAAACTTTCCACGTGAACCGTATCAACCCCTTAAATTTGGGG
GCTTGTTCGGCGGCGTGCGTGCGGGCCTGTAAGGGGTCGGCGTGCTGCTGCTGGGCGGGCTCTATCAGGGGCG
AGCGTTTGGGCTTCGGCTCGAGCTAGTAGCTATCACTTTTAAACTCCTTTCTTAAAATACTGAACATACTGTGG
GGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGA
ACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATATTGCA
CTTCCGGGTTAGTCCTGGGAGTATGCCTGTATCAGTGTCCGTACATCAACCTTGGTTTTCTTCCTTCCGTGTAGT
CGGTGGAGGATATGCCAGACGTGAAGTGTCTTGCTAGCGGTCTTTCGAGTCTGCGTGTGAGTCCTTTGAAATGT

ACTGAACTGTACTCTCTCTTTGTCCGAAAAGCGTGGCGTTGCTGGTTGTGGAGGCTGCCCGTGTGGCCAGTCGG
CGACCGGTTTGTTAGCTGTGGCGTTTAATGGAGGAGTGTTCGATTCGCGGTATGGTTGGCTTCGGCTGAACAAT
CTGCTTATTGGGTGCTTTTCCTGTCATTGGCGGTACGAACTGGTGAACCGTAGCTATGTGGTGCTTGGCTTTTGA
ACCGGCTTTGCTTTGCGAAGTAGGGTGACAGTTTCGGCTGTCGAGGGGTTGATCCA

T24

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T28

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T29

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T33

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T44

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T45

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T52

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T58

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T65

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T73

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T95

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T101

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T113

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T125

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T128

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LIF 13

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LIF 16

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LIF 18

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AAAT

LIF 19

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GGTGAACCTGCGGAAGGATCATTACCACACCTAAAAAACTTTCCACGTGAACCGTATCAACCCCTATAATTCG
GGGGCTTGCTCGGCGGCGTGCGTGCTGGCCTGTAATGGGTCGGCGTGCTGCTGCTGGGCGGGCTCTATCATGG
GCGAGCGCTTGGGCTTCGGCTCGAGCTAGTAGCTTTTTCTTTTAAACCCATTCTTTAAATACTGAACATACTGTG
GGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAG
AACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATATTGC
ACTTCCGGGTTAGTCCTGGGAGTATGCCTGTATCAGTGTCCGTACATCAACCTTGGTTTTCTTCCTTCCGTGTAG
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TACTGAACTGTACTCTCTCTTTGCTCGAAAAGCGTGGCGTTGCTGGTTGTGGAGGCTGCCCGTGTGGCCAGTCG
GCGACCGGTTCGTTAGCTGTGGCGTTTAATGGAGGAGTGTTCGATTCGCGGTATGGTTGGCTTCGGCTGAACAA
TCTGCTTATTGGGTGCTTTTCCTGCTGCAGCGGTATGAACTGGTGAACCGTAGCTGTGTGGTGCTTGGCTTTTGA
ACCGGCTTTGCTTTGCGAAGTAGAGTGGCGGCTTCGGCTGTCGAGGGGTCGATCCATTTTGGGAAACTTTGTGT
GTGCGGCTTCGTGCTGCGCGCATCTCAATTGGACCTGATATCAGGCAAGATTACCCGCTGAACTTAAGCATATC
AATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGCGGGAAGAGCTCAAGCTT
AAAA

LIF 42

ATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGACTCGGTGAAAAATTGGGACCG
TGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCGCCATTTAGAGGAAGGTGAAGT
CGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAAAAACTTTCCACGTGAACCGT
ATCAACCCCTATAATTCGGGGGGCTTGCTCGGCGGCGTGCGTGCTGGCCTGTAATGGGTCGGCGTGCTGCTGCTG
GGCGGGCTCTATCATGGGCGAGCGCTTGGGCTTCGGCTCGAGCTAGTAGCTTTTTCTTTTAAACCCATTCTTTAA
ATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGC
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TCTTCCTTCCGTGTAGTCGGTGGAGGATATGCCAGACGTGAAGTGTCTTGCTGGCGGTCTTTCGAGTCTGCCGG
TGAGTCCTTTGAAATGTACTGAACTGTACTCTCTCTTTGCTCGAAAAGCGTGGCGTTGCTGGTTGTGGAGGCTG
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GGCTTCGGCTGAACAATCTGCTTATTGGGTGCTTTTCCTGCTGCAGCGGTATGAACTGGTGAACCGTAGCTGTG
TGGTGCTTGGCTTTTGAACCGGCTTTGCTTTGCGAAGTAGAGTGGCGGCTTCGGCTGTCGAGGGGTCGATCCAT
TTTGGGAAACTTTGTGTGTGCGGCTTCGTGCTGCGCGCATCTCAATTGGACCTGATATCAGGCAAGATTACCCG
CTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAG

LIF 89

CGCAGTCATCAGCTTGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGA
CTCGGTGAAAAATTGGGACCGTGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCG
CCATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAA
AAAAACTTTCCACGTGAACCGTATCAACCCCTTTAATTTGGGGGCTTGTTCGGCGGCGTGCGTGCTGGCCTGTA
ATGGGTCGGCGTGCTGCTGCTGGGCGGGCTCTATCATGGGCGAGCGTTTGGGCTTCGGCTCGAGCTAGTAGCTA
TCACTTTTAAACTCCTTTCTTAAAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCA
ACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGACTTGCACGGAGATTTTAAGCTTGAGCTCTTCCCGCT
TCACTCGCCGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTA
ATCTTGCCTGATATCAGGTCCAATTGAGATGCACGCAGCACGAAGCCGCACACACAAAGTTTCCCAAAATGGA
TCGACCCCTCGACAGCCGAAACTGTCACCCTACTTCGCAAAGCAAAGCCGGTTCAAAAGCCAAGCACCACATA
GCTACGGTTCACCAGTTCGTACCGCCAATGACAGGAAAAGCACCCAATAAGCAGATTGTTCAGCCGAAGCCAA
CCATACCGCGAATCGAACACTCCTCCATTAAACGCCACAGCTAACAAACCGGTCGCCGACTGGCCACACGGGC
AGCCTCCACAACCAGCAACGCCACGCTTTTCGGACAAAGAGAGAGTACAGTTCAGTACATTTCAAAGGACTCA
CACGCAGACTCGAAAGACCGCTAGCAAGACACTTCACGTCTGGCATATCCTCCACCGACTACAC

LIF 98

CGCAGGTCATCAGCTTGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATG
ACTCGGTGAAAAATTGGGACCGTGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTC
GCCATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTA
AAAAAACTTTCCACGTGAACGGTATCACCCCCTTAAATTTGGGGGCTTGCTCGGGGGCGGGCGTCCGGCCCTGT
AAGGGGCCGGCGTGCTGCTGCGGGGCTTGCACGGAGATTTTAAGCTTGAGCTCTTCCCGCTTCACTCGCCGTTA
CTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAATCTTGCCTGATA
TCAGGTCCAATTGAGATGCGCGCAGCACGAAGCCGCACACACAAAGTTTCCCAAAATGGATCGACCCCTCGAC
AGCCGAAGCCGCCACCCTACTTCGCAAAGCAAAGCCGGTTCAAAAGCCAAGCACCACATAGCTACGGTTCACC
AGTTCGTACCGCCAATGACAGGAAAAGCACCCAATAAGCAGATTGTTCAGCCGAAGCCAACCATACCGCGAAT
CGAACACTCCTCCATTAAACGCCACAGCTAACAAACCGGCGCGACTG

LIF 109

CGCAGTCATCAGCTTGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGA
CTCGGTGAAAAATTGGGACCGTGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCG
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TCACTTTTAAACTCCTTTCTTAACTTGCACGGAGATTTTAAGCTTGAGCTCTTCCCGCTTCACTCGCCGTTACTA
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GGTCCAATTGAGATGCACGCAGCACGAAGCCGCACACACAAAGTTTCCCAAAATGGATCGACCCCTCGACAGC
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TCGTACCGCCAATGACAGGAAAAGCACCCAATAAGCAGATTGTTCAGCCGAAGCCAACCATACCGCGAATCGA
ACACTCCTCCATTAAACGCCACAGCTAACAAACCGGTCGCCGACTGGCCACACGGGCAGCCTCCACAACCAGC
AACGCCACGCTTTTCGGACAAAGAGAGAGTACAGTTCAGTACATTTCAAAGGACTCACACGCAGACTCGAAAG
ACCGCTAGCAAGACACTTCACGTCTGGCATATCCTCCACCGACTACACGGAAGGAAGAAAACCAAGGTTGATG
TACGGACACTGATACAGGCATACT

LIF 180

GCAGTCATCAGCTTGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGAC
TCGGTGAAAAATTGGGACCGTGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCGC
CATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAA
AAAACTTTCCACGTGAACCGAATCAACCCTTTAAATTTGGGGGCTTGTTCGGCGGCGTGCGTGCGGGCCTGTAA
GGGGCCGGCGTGCTGCTGCGGGGCGGGCTCTATCAGGGGCAAGCGTTTGGCCTTCGGCTCGAGCTAGTAGCTA
TCACTTTTAAACTCTTTTCTAAACTTGCACGGAGATTTTAAGCTTGAGCTCTTCCCGCTTCACTCGCCGTTACTA
GGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAATCTTGCCTGATATCA
GGTCCAATTGAGATGCACGCAGCACGAAGCCGCACACACAAAGTTTCCCAAAATGGATCGACCCCTCGACAGC
CGAAGCTGCCACCCTACTTCGCAAAGCAAAGCCGGTTCAAAAGCCAAGCACCACATAGCTACGGTTCACCAGT
TCGTACCGCCAATGACAGGAAAAGCACCCAATAAGCAGATTGTTCAGCCGAAGCCAACCATACCGCGAATCGA
ACACTCCTCCATTAAACGCCACAGCTAACAAACCGGTCGCCGAC

LIF 192

GCTAGTCATCAGCTTGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGA
CTCGGTGAAAAATTGGGACCGTGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCG
CCATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAA
AAAAACTTTCCACGTGAACCGTATCAACCCCTTTAATTTGGGGGCTTGTTCGGCGGCGTGCGTGCTGGCCTGTA
ATGGGTCGGCGTGCTGCTGCTGGGCGGGCTCTATCATGGGCGAGCGTTTGGGCTTCGGCTCGAGCTAGTAGCTA
TCACTTTTAAACTCCTTTCTTAAAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCA
ACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTG
CAGGATTCAGTGAGTCATCGAAATTTTGAACGCAT

LIF 213

TTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGACTCGGTGAAAAATTGGGA
CCGTGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCGCCATTTAGAGGAAGGTGA
AGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAAAAAAACTTTCCACGTGAA
CGGTATCACCCCCTTAAATTTGGGGGCTTGCTCGGGGGCGGGCGTCCGGCCCTGTAAGGGGCCGGCGTGCTGC
TGCGGGGCGGGCTCTATCATGGGCGAGCGTTTGGGCTTCGGCTCGAACTAGTAGCTATCAATTTTAAACCCTTT
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TGCGGCTTCGTGCTGCGCGCATCTCAATTGGACCTGATATCAGGCAAGATTACCCGCTGAACTTAAGCATATCA
ATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGCGGGAAGAGCTCAAGCTTA
AAA

LIF 224

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CTCGGTGAAAAATTGGGACCGTGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCG
CCATTTAGAGGAAGGTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAA
AAAAACTTTCCACGTGAACCGTATCAACCCCTTTAATTTGGGGGCTTGTTCGGCGGCGTGCGTGCTGGCCTGTA
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TCACTTTTAAACTCCTTTCTTAAAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCA
ACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTG
CAGGATTCAGTGAGTCATCG

LIF 231

CGCAGTCATCAGCTTGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGA
CTCGGTGAAAAATTGGGACCGTGAGTCCGTTTGCTTCATTGCGAGTGGATTGATGGGAACTTTTTTAAACCTCG
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AAAAACTTTCCACGTGAACCGTATCAACCCCTTTAATTTGGGGGCTTGTTCGGCGGCGTGCGTGCTGGCCTGTA
ATGGGTCGGCGTGCTGCTGCTGGGCGGGCTCTATCATGGGCGAGCGTTTGGGCTTCGGCTCGAGCTAGTAGCTA
TCACTTTTAAACTCCTTTCTTAAAATACTGAACATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCA
ACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTG
CAGGATTCAGTGAGTCATCGAAATTTTGAACGCATA

coxI **sequences for** ***Phytophthora*** **isolates**

T3

GGAGGTTTTGGTAACTGGTTTGTTCCTTTAATGATAGGTGCCCCTGATATGGCTTTTCCACGTATGAATAATATA
AGTTTTTGGTTATTACCTCCAGCTTTATTATTATTAGTTTCATCAGCTATTGTAGAATCTGGTGCTGGTACAGGT
TGGACTGTTTATCCACCATTATCTAGTGTACAAGCACATTCAGGACCTTCAGTAGATTTAGCTATTTTTAGTTTA
CATTTAACAGGTATTTCTTCATTATTAGGTGCAATTAATTTTATTTCAACTATTTATAATATGAGAGCTCCTGGT
TTAAGTTTTCATAGATTACCTTTATTTGTTTGGTCAGTATTAATTACAGCATTTCTTTTATTATTAACTTTACCTG
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AGCTTATTTTTCAGCAGCTACTATGATTATTGCTGTACCTACTGGTATTAAAATTTTCAGTTGGTTAGCTACTTT
ATGGGGTGGTTCTTTAAAATTTGAAACTCCTTTATTATTTACTTTAGGATTTATTTTATTATTCGTTATGGGTGGT
GTAACTGGAGTAGCTATGTCAAAC

T5

TTAGCACAACCCGGTAATCAAATCTTTATGGGAAATCATCAATTATATAACGTTATTGTTACTGCTCACGCTTTT
ATTATGGTTTTCTTTTTAGTTATGCCTGCTTTAATTGGAGGTTTTGGTAACTGGTTTGTTCCTTTAATGATAGGTG
CCCCTGATATGGCTTTTCCACGTATGAATAATATAAGTTTTTGGTTATTACCTCCAGCTTTATTATTATTAGTTTC
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GGTGCACCTATCATTAAAGGAACAAACCAGTTACCAAAACCACCTATTAAAGCAGGCATAACTAAAAAGAAA
ACCATAATAAAAGCATGTGCAGTAACAACAACATTATATAATTGATGATTTCCCATAAAAATTTGATTACCTGG

T6

AGCACAACCCGGTAATCAAATCTTTATGGGAAATCATCAATTATATAACGTTATTGTTACTGCTCACGCTTTTA
TTATGGTTTTCTTTTTAGTTATGCCTGCTTTAATTGGAGGTTTTGGTAACTGGTTTGTTCCTTTAATGATAGGTGC
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TCAGCTATTGTAGAATCTGGTGCTGGTACAGGTTGGACTGTTTATCCACCATTATCTAGTGTACAAGCACATTC
AGGACCTTCAGTAGATTTAGCTATTTTTAGTTTACATTTAACAGGTATTTCTTCATTATTAGGTGCAATTAATTT
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CTTTAGGATTTATTTTATTATTCGTTATGGGTGGTGTAACTGGAGTAGCTATGTCAAACTCAGGTTTAGATATTG
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T8

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CATAATGGAAATGACCTACAATATAATAAGTATCATGGATTGCAATATCTAAACCTGAATTAGACATAGCTAC
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CTGAAAAATAAGCTCTAGTATCTACATCTAAACCAACAGTAAACATGTGGTGTGCCCAAACAATACAACCTAA
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T24

TTTTTCCAATCCAAAAATAAAATCCAGTAAATATACCAAAAACGGCACCCATAGATAATACATAATGGAAATG
TCCTACAATATAATATGTATCATGAATTGCAATATCTAAACCTGAATTAGACATTGCTACTCCGGTTACACCAC
CCATAACAAATAATAAAATAAAACCTAAAACAAATAATAAAGGTGTTTCAAATTTTAAAGAACCACCCCATAA
AGTAGCTAACCAACTAAAAATTTTAATACCAGTAGGTACAGCAATAATCATAGTCGCTGCTGAGAAATAAGCT
CGAGTATCTACATCTAAACCAACAGTAAACATATGATGTGCCCAGACAATACAACCTAATAAACCAATAGATA

ACATTGCATAAACCATTCCTAAATAACCGAATACATTTTTTTTAGCAAAAGCTGCGGCAACTTGACTAATAATA
CCAAAAGCAGGTAAAATTAAAATATAAACTTCAGGATGACCGAAAAACCAAAATAAATGTTGATATAATACC
GGATCACCTCCTCCAGATGGATCGTAAAACGAAGTATTTAAATTTCTATCAGTTAATAACATAGTAATGGCACC
AGCTAATACGGGTAATGTTAATAATAAAAGAAAAGCTGTAATTAACACAGACCAAACAAATAGGGGTAATCT
GTG

T28

AATTATATAACGTTATTGTTACTGCTCACGCTTTTATTATGGTTTTCTTTTTGGTTATTCTTTATTGGTGAGGAGG
TTTTGGTAACTGGTTTGTTCCTTTAATGATAGGTGCCCCTGATATGGCTTTTCCACGTATGAATAATATAAGTTT
TTGGTTATTACCTCCAGCTTTATTATTATTAGTTTCATCAGCTATTGTAGAATCTGGTGCTGGTACAGGTTGGAC
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GGTGGTTCTTTAAAATTTGAAACTCCTTTATTATTTACTTTAGGATTTATTTTATTATTCGTTATGGGTGGTGTAA
CTGGAGTAGCTATGTCAAACTCAG

T29

CCAATTATATAATGTTATTGTTACAGCGCATGCTTTTATTATGGTTTTCTTTTTAGTTATGCCAGCCCTAATAGG
TGGTTTTGGTAATTGGTTTGTTCCTTTAATGATAGGGGCACCTGATATGGCTTTTCCACGTATGAATAATATTAG
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TTTAACAGGTATTTCATCACTATTAGGTGCGATTAATTTTATTTCAACTATTTATAATATGAGAGCTCCTGGTTT
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GTAACAGGTGTAGCTATGTCTAATTCAGGTTTAGATATTGCAATCCATGATACTTATTATATTGTAGGTCATTTC
CATTATGTATTATCAATGGGTGCTGTTTTT

T32

TTTTTATGGGAAATCACCAATTATATAATGTTATTGTTACAGCGCATGCTTTTATTATGGTTTTCTTTTTAGTTAT
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TATGAATAATATTAGTTTTTGGTTATTACCACCAGCTTTATTATTATTAGTGTCATCAGCTATTGTTGAATCAGG
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GAGAGCTCCTGGTTTAAGTTTCCATAGATTACCTTTATTTGTTTGGTCTGTATTAATTACAGCATTTCTTTTATTA
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CCATCTGGTGGGGGAGATCCCGTATTATATCAACATTTATTTTGGTTTTTTGGTCACCCTGAAGTTTATATTTTA
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TGGTTAGCTACTTTATGGGGTGGTTCATTAAAATTTGAAACACCTTTATTATTTGTTTTAGGTTTTATCTTATTAT
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T33

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TATCTATTGGATTATTAGGTTCAATTGTTTGGGCACACCATATGTTTACTGTTGGTTTAGATGTAGATACTCGAG
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GGGGTGGTTCTTTAAAATTTGAAACTCCTTTATTATTTACTTTAGGATTTATTTTATTATTCGTTATGGGTGGTGT
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T35

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T43

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T45

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T46

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T52

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T58

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T65

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GGA

T88

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T95

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T100

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T113

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LIF 13

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C

LIF 18

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LIF 42

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LIF 89

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LIF 109

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LIF 180

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LIF 213

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CATTATGTATTATCAATGGGTGCTGTTTTTGGTATATTTACTGGA

A.2.6

Table A1: One-way ANOVA results for mean number of *Phytophthora* isolates Vs baiting method (i.e., laboratory baiting and river baiting)

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
Baiting method	1	38.9	38.89	1.819	0.189
residual	26	555.8	21.38		

Table A2: One-way ANOVA results for mean number of *Phytophthora* spp. Vs baiting method (i.e., laboratory baiting and river baiting)

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
Baiting method	1	2.286	2.286	2	0.169
residual	26	29.714	1.143		

Table A3: One-way ANOVA results for mean number of *Phytophthora* isolates in laboratory baiting Vs leaf bait types

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
Bait type	6	56.43	9.405	3.559	0.0606
residual	7	18.50	2.643		

Table A4: One-way ANOVA results for mean number of *Phytophthora* isolates in river baiting Vs leaf bait types

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
Bait type	6	389.9	64.98	4.998	0.0266
residual	7	91	13		

Table A5: One-way ANOVA results for mean number of *Phytophthora* isolates in river baiting and laboratory baiting Vs leaf bait types.

Source of variation	d.f	s.s	m.s	F value	P value
Bait type	6	325.4	54.24	4.23	0.00605
residual	21	269.2	12.82		

Table A6: Tukey's honestly significant difference (HSD) for the number of *Phytophthora* isolates Vs bait types. Significant comparisons (P=0.05) are in bold.

Linear hypothesis	Estimate std.	Error	t value	P value
Ca. japonica - B. attenuata == 0	-1.75	2.532	-0.691	0.9917
Ce. deodara - B. attenuata == 0	0.25	2.532	0.099	1
Pt. eugenioides – B. attenuata == 0	-1	2.532	-0.395	0.99963
Pi. radiata - B. attenuata == 0	2	2.532	0.79	0.98347
Pt. undulatum - B. attenuata == 0	0.75	2.532	0.296	0.99993
R. arboreum - B. attenuata == 0	9.25	2.532	3.653	**0.0213***
Ce. deodara - Ca. japonica == 0	2	2.532	0.79	0.98348
Pt. eugenioides - Ca. japonica == 0	0.75	2.532	0.296	0.99993
Pi. radiata - Ca. japonica == 0	3.75	2.532	1.481	0.75236
Pt. undulatum - Ca. japonica == 0	2.5	2.532	0.987	0.95129
R. arboreum - Ca. japonica == 0	11	2.532	4.344	**0.00452****
Pt. eugenioides - Ce. deodara == 0	-1.25	2.532	-0.494	0.99868
Pi. radiata - Ce. deodara == 0	1.75	2.532	0.691	0.99172
Pt. undulatum - Ce. deodara == 0	0.5	2.532	0.197	0.99999
R. arboreum - Ce. deodara == 0	9	2.532	3.555	**0.0262***
Pi. radiata - Pt. eugenioides == 0	3	2.532	1.185	0.89216
Pt. undulatum - Pt. eugenioides == 0	1.75	2.532	0.691	0.99171
R. arboreum - Pt. eugenioides == 0	10.25	2.532	4.048	**0.00871****
Pt. undulatum - Pi. radiata == 0	-1.25	2.532	-0.494	0.99868
R. arboreum - Pi. radiata == 0	7.25	2.532	2.863	0.10807
R. arboreum - Pt. undulatum == 0	8.5	2.532	3.357	**0.04025***

* significantly different (P≤0.05), **significantly different (P≤0.005).

Table A7: One-way ANOVA results for mean number of *Phytophthora* spp. in laboratory baiting Vs leaf bait types

Source of variation	d.f	s.s	m.s	F-value	P-value
Bait type	6	4.857	0.8095	2.833	0.0996
residual	7	2.000	0.2857		

Table A8: One-way ANOVA results for mean number of *Phytophthora* spp. in river baiting Vs leaf ba it types

Source of variation	d.f	s.s	m.s	F-value	P-value
Bait type	6	14.86	2.476	2.167	0.167
residual	7	8	1.143		

Table A9: One-way ANOVA results for mean number of *Phytophthora* spp. in river baiting and labora tory baiting Vs leaf bait types

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
Bait type	5	9.833	1.9667	2.023	0.124
residual	18	17.500	0.9722		

Appendix A.3: Supplementary material for chapter 3

A.3.1

Table A1: GPS coordinates for the 25 sites sampled in six Canterbury waterways in May 2018.

River	site	GPS Latitude	GPS Longitude	Salinity ppt	pH	Water temp °C	NH4-N (mg/L)	NO_3-N (mg/L)
Lake Hood	1	-43.972413958981633	171.7621379904449	0.2	7.2	9	0.11	1.24
Ashburton	1	-43.732418026775122	171.59085497260094	0.2	7.6	8.5	0.17	0.19
Ashburton	2	-43.741669971495867	171.5290039870888	0.1	7.0	11	0.08	0.12
Ashburton	3	-43.631066987290978	171.30136800929904	0.1	7.1	7	0.08	0.15
Ashburton	4	-43.625611960887909	171.3025080319494	0.1	7.2	4	0.08	0.03
Ashburton	5	-43.812863007187843	171.68677403591573	0.1	7.2	4	0.07	0.03
Ashburton	6	-44.000706989318132	171.78675597533584	0.1	7.0	7.5	0.09	0.52
Selwyn (Coes ford)	1	-43.695950964465737	172.41291599348187	0.0	7.0	9	0.07	1.23
Selwyn	2	-43.664256976917386	172.3149390053004	0.2	7.2	9	0.10	5.37
Selwyn	3	-43.688170965760946	172.37209201790392	0.1	7.1	11	0.08	7.36
Selwyn	4	-43.717193976044655	172.43950095959008	0.1	7.2	10	0.07	5.77
Selwyn	5	-43.455639034509659	171.87322997488081	0.1	7.2	9	0.08	5.17
Selwyn	6	-43.461421960964799	171.89307999797165	0.0	6.7	4	0.05	0.59
Selwyn	7	-43.551416024565697	172.02897101640701	0.0	7.3	7	0.06	0.51
Selwyn	8	-43.646427998319268	172.23351401276886	0.0	7.3	9	0.07	0.67
Prices Valley	1	-43.760222224518657	172.72636111825705	0.0	7.2	12	0.04	1.48
Prices Valley	2	-43.762289034202695	172.72211995907128	0.1	7.0	8	0.07	0.24
Prices Valley	3	-43.767409035935998	172.71176503971219	0.1	7.3	9	0.07	0.01
Prices Valley	4	-43.793436018750072	172.68478796817362	0.0	7.0	9	0.07	0.21
Kaituna Valley	1	-43.774557039141655	172.65624498948455	0.0	7.0	9	0.08	0.15
Kaituna Valley	2	-43.742505982518196	172.68731997348368	0.2	7.0	8	0.05	0.09

Table A1 continued.

River	site	GPS Latitude	GPS Longitude	Salinity ppt	PH	Wat-er temp °C	NH4-N (mg/L)	NO_3-N (mg/L)
Kaituna Valley (Okana stream)	3	-43.750754026696086	172.68928502686322	0.0	7.6	8	0.06	0.07
Halswell	1	-43.661992019042373	172.54232998006046	0.1	7.9	10	0.10	2.84
Halswell	2	-43.656850978732109	172.51537696458399	0.1	7.6	11	0.14	2.88
Halswell	3	-43.751447964459658	172.60461104102433	0.2	7.8	10	0.23	0.16

A.3.1

Table A3: Master mix for amplification of the β-tubulin gene using TUBUF2 and TUBR1 primers.

Component	Final concentration	Final volume used
DNA	10 ng/ µL	2 µl
Dream Taq	1 unit	10 µl
TUBU F2 10 µM stock	0.5µM	1 µl
TUBUR1 10 µM stock	0.5µM	1 µl
Deionised water		6 µl

A.3.2 DNA sequence of *Phytophthora* isolates: ITS, *coxI* and β tubulin

ITS sequence for *Phytophthora* isolates

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TACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGACTCGGTGAAAAATTGGGACCGTG
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267

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271

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310

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324

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352

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367

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395

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400

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412

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427

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435

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CGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAA
GCGGGAAGAGCTCAAGCTTAAAATCTCCGTGCAAG

coxI sequence for *Phytophthora* isolates

279

TTTTCCAATCCAAAAATAAAATCCAGTAAAGATACCAAAAACAGCACCCATAGATAATACATAATGGAAATGT
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AAAGCTGGTAAAATTAAAATATAAACTTCAGGATGACCAAAAAACCAAAATAAATGTTGATATAATACGGGAT
CACCTCCTCCTGAAGGATCATAGAAAGATGTATTTAAGTTTCTATCAGTTAATAACATAGTAATGGCACCAGCT
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280

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ATACA

291

AATAAAACCTAAAACAAATAATAAAGGTGTTTCAAATTTTAAAGAACCTCCCCATAAAGTTGCTAACCAACTA
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GGATCATAAAAAGAAGTATTTAAATTTCTATCAGTTAATAACATTGTAATGGCACCAGCTAATACAGGTAATGT
TAATAATAAAAGAAATGCTGTAATTAATACAGACCAAACAAATAAAGGTAATCT

292

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316

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328

ACAACCAGGTAATCAAATTTTTATGGGAAATCATCAATTATATAATGTTATTGTTACTGCACACGCTTTTATTAT
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352

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361

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382

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CTAGTATCTACATCTAAACCAACAGTAAACATGTGGTGTGCCCAAACAATACAACCTAATAAACCTATTGATA
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395

TTTTTCCAATCCAAAAATAAAATCCAGTAAATATACCAAAAACAGCACCCATTGATAATACATAATGGAAATG
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416

TAATCAAATTTTTATGGGAAATCACCAATTATATAATGTTGTTGTTACTGCACATGCTTTTATTATGGTTTTCTTT
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463

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ACTATTTATAATATGAGAGCTCCTGGTTTAAGTTTTCACAGATTACCCCTATTTGTTTGGTCTGTGTTAATTACA
GCTTTTCTTTTATTATTAACATTACCCGTATTAGCTGGTGCCATTACTAT

β tubulin sequence for *Phytophthora* isolates

279

GTCGGACACGGTCGTGGAACCCTACAACGCCACGCTGTCGGTGCACCAGCTGGTCGAGAACGCCGATGAGGTC
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289

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GTCCGAGCAGTTCACGGCCATGTTCCGTCGTAAGGCTTTCTTGCACTGGTACACTGGCGAGGGTATGGATGAGA
TGGAGT

309

CGTCCATACCCTCGCCAGTGTACCAGTGCAAGAAAGCCTTACGACGGAACATGGCCGTGAACTGCTCGGACAC
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ACGGTACTGCTGCGAGCCGCGCGACGTCAG

324

CCAGATCACGCACTCGCTCGGCGGTGGTACCGGCTCCGGTATGGGAACGCTTCTTATCTCGAAGATCCGTGAA
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325

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328

TGTGACTGCCTGCAGGGTTTCCAGATTACGCACTCGCTCGGCGGTGGTACCGGCTCCGGTATGGGAACGCTTCT
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382

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416

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483

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CGGTAACTCGACCGCCATCCAGGAGATGTTCAAGCGCGTGTCCGAGCAGTTCACGGCCATGTTCCGTCGTAAG
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β tubulin sequence for *Pythium* isolates

273

GCTGTGACTGCCTCCAGGGCTTCCAGATCACCCACTCGCTTGGTGGTGGTACTGGTTCGGGTATGGGTACCCTT
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ACTGGTACA

278

TCGACGTCGTCCGCAAGGAGGCTGAGAGCTGTGACTGCCTCCAGGGCTTCCAGATCACCCACTCGCTTGGTGGT
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GTCGTACTTCGTCGAGTGGATCCCGAACAACATCAAGGCCAGCGTTTGTGACATCCCTCCAAAGGGCCTCAAG
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322

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385

GTCGGGCATCACCACGTGTCTGCGTTTCCCAGGTCAGCTGAACTCGGATCTCCGCAAGCTCGCCGTGAACTTGA
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CGGTCGCTACCTCACCGCCGCGTGTATGTTCCGTGGCCGCATGAGCACCAAGGAAGTCGATGAGCAGATGCTC
AACGTGCAGAACAAGAACTCGTCGTACTTCGTCGAGTGGATCCCGAACAACATCAAGGCCAGCGTTTGTGACA
TCCCTCCAAAGGGTCTCAAGATGAGCACGACCTTCGTCGGTAACTCGACGGCGATCCAGGAGATGTTCAAGCG
TGTGAGCGAGCAGTTCACGGCCATGTTCCGTCGTAAGGCCTTCTTGCACTGGTACACTGGTGAGGGCATGGAC
GAGATGGAGTTCACTGAAGCCGAGTCGAACATGAACGATCTGGTGTCTGAGTACAGCACGATAGCAGGTGAA
AATTGGCGCGAGTCACGCCCGGGTCGCGACGTGTTCTAGAGCTGCAGTC

386

TTTCATCCATACCCTCACCCGTGTACCAGTGCAAGAAAGCCTTGCGACGGAACATGGCCGTGAACTGCTCGGA
CACACGCTTGAACATCTCCTGGATGGCAGTCGAGTTACCGATGAACGTGGTACTCATCTTGAGGCCCTTAGGCG
GGATGTCACACACGCTGGCCTTGATGTTGTTGGGGATCCATTCAACGAAGTACGAGGAGTTCTTGTTCTGCACG
TTCAGCATCTGTTCATCGACTTCCTTGGTACTCATGCGGCCGCGGAACATACAGGCAGCAGTTAAATAACGACC
GTGGCGGGGATCAGCAGCGCACATCATATTCTTGGCGTCAAACTGCTGCTGGGTCAGCTCAGGGACGGTGAGA
GCACGGTACTGCTGCGAGCCACGAGAGGTCAGAGGCGCGAAACCAATCATGAAGAAGTGGAGGCGCGGGAAA
GGAATCAGGTTCACGGCCAGCTTACGCAGATCGGAGTTCAACTGACCCGGGAAACGCAGGCACGTCGTGAT

414

GGAGGCTGAGAGCTGTGACTGCCTCCAGGGCTTCCAGATCACCCACTCGCTTGGTGGTGGTACTGGTTCGGGTA
TGGGTACCCTCTTTGATCTCCAAGATCCGTGAAGAATACCCAGACCGTATCATGTGCACGTACTCGGTGTGCCC
GTCGCCAAAGGTGTCGGACACTGTCGTGGAGCCTTACAACGCCACGCTCTCGGTGCACCAGCTCGTCGAGAAC
GCCGATGAGGTCATGTGCCTGGACAACGAAGCCCTCTACGATATCTGCTTCCGTACGCTCAAGCTCACGACCCC

AACCTACGGTGACCTGAACCACCTCGTGTGTGCCGCCATGTCGGGCATCACCACGTGTCTGCGTTTCCCAGGTC
AGCTGAACTCGGATCTCCGCAAGCTCGCCGTGAACTTGATCCCGTTCCCGCGTCTCCACTTCTTCATGATCGGTT
TCGCCCCACTGACGTCGCGTGGCTCGCAGCAGTACCGTGCCCTCACGGTCCCAGAGCTGACCCAGCAGCAGTT
CGACGCCAAGAACATGATGTGCGCCGCCGATCCTCGCCACGGTCGCTACCTCACCGCCGCGTGTATGTTCCGTG
GCCGCATGAGCACCAAGGAAGTCGATGAGCAGATGCTCAACGTGCAGAACAAGAACTCGTCGTACTTCGTCGA
GTGGATCCCGAACAACATCAAGGCCAGCGTTTGTGACATCCCTCCAAAGGGCCTCAAGATGAGCACGACCTTC
GTCGGTAACTCGACGGCGATCCAGGAGATGTTCAAGCGTGTGAGCGAGCAGTTCACGGCCATGTTCCGTCGTA
AGGCCTTCTTGCACTGGTACACCGGTGAGGGCATGGACGAGATGGAGT

424

GACGTCGTCCGTAAGGAGGTTGAGAGCTGTGACTGCCTCCAGGGCTTCCAGATCACCCACTCGCTTGGTGGTG
GTACTGGTTCGGGTATGGGTACCCTTTTGATCTCCAAGATCCGTGAAGAATACCCAGACCGTATCATGTGCACG
TACTCGGTGTGCCCGTCGCCAAAGGTGTCGGACACCGTCGTGGAGCCTTACAACGCCACGCTCTCGGTGCACC
AGCTCGTCGAGAACGCCGATGAGGTCATGTGCCTGGACAACGAAGCCCTCTACGATATCTGCTTCCGTACGCTC
AAGCTCACGACCCCAACCTACGGTGACCTGAACCACCTCGTGTGTGCCGCCATGTCGGGCATCACCACGTGTCT
GCGTTTCCCAGGTCAGCTGAACTCGGATCTCCGCAAGCTCGCCGTGAACTTGATCCCGTTCCCGCGTCTCCACT
TCTTCATGATCGGTTTCGCCCCACTGACGTCGCGTGGCTCGCAGCAGTACCGTGCCCTCACGGTCCCAGAGCTG
ACCCAGCAGCAGTTCGACGCCAAGAACATGATGTGCGCCGCCGATCCTCGCCACGGTCGCTACCTCACCGCCG
CGTGTATGTTCCGTGGCCGCATGAGCACCAAGGAAGTCGATGAGCAGATGCTCAACGTGCAGAACAAGAACTC
GTCGTACTTCGTCGAGTGGATCCCGAACAACATCAAGGCCAGCGTTTGTGACATCCCTCCAAAGGGCCTCAAG
ATGAGCACGACCTTCGTCGGTAACTCGACGGCGATCCAGGAGATGTTCAAGCGTGTGAGCGAGCAGTTCACGG
CCATGTTCCGTCGTAAGGCCTTCTTGCACTGGTACACCGGTGAGGGCATGGACGAGATGGAGTCAC

458

TCCTGTGATTGCCTGCAGGGTTTCCAGATTACGCACTCCCTCGGTGGTGGTACTGGTTCCGGTATGGGCACGCT
TCTGATCTCTAAGATCCGTGAAGAGTACCCGGATCGTATCATGTGCACGTACTCGGTTTGCCCTTCGCCCAAGG
TGTCGGATACCGTCGTGGAGCCCTACAATGCCACGCTATCGGTCCACCAGCTCGTCGAAAACGCTGATGAAGT
CATGTGCCTTGACAACGAAGCCCTCTACGATATTTGCTTCCGTACGCTCAAGCTGACCACGCCTACCTACGGTG
ATCTGAACCACCTGGTATGCGCTGCCATGTCGGGTATCACGACGTGCCTGCGTTTCCCGGGTCAGTTGAACTCC
GATCTGCGTAAGCTGGCCGTGAACCTGATTCCGTTCCCGCGCCTCCACTTCTTCATGATTGGTTTCGCGCCTCTG
ACCTCTCGTGGCTCGCAGCAGTACCGTGCTCTCACCGTCCCTGAGCTGACCCAGCAGCAGTTTGACGCCAAGAA
CATGATGTGCGCTGCTGATCCCCGCCACGGTCGCTATTTAACTGCTGCCTGTATGTTCCGCGGCCGCATGAGTA
CCAAGGAAGTCGATGAACAGATGCTGAACGTGCAGAACAAGAACTCCTCGTACTTCGTTGAATGGATCCCCAA
CAACATCAAGGCCAGCGTGTGTGACATCCCGCCTAAGGGTCTCAAGATGAGTACCACGTTCATCGGTAACTCG
ACTGCCATCCAGGAGATGTTCAAGCGTGTGTCCGAGCAGTTCACGGCCATGTTCCGTCGCAAGGCTTTCTTGCA
CTGGTACACGGGTGAGGGTATGGATGAAA

460

GTGTACCAGTGCAAGAAGGCCTTACGACGGAACATGGCCGTGAACTGCTCGCTCACACGCTTGAACATCTCCT
GGATCGCCGTCGAGTTACCGACGAAGGTCGTGCTCATCTTGAGGCCCTTTGGAGGGATGTCACAAACGCTGGC
CTTGATGTTGTTCGGGATCCACTCGACGAAGTACGACGAGTTCTTGTTCTGCACGTTGAGCATCTGCTCATCGA
CTTCCTTGGTGCTCATGCGGCCACGGAACATACACGCGGCGGTGAGGTAGCGACCGTGGCGAGGATCGGCGGC
GCACATCATGTTCTTCGCGTCGAACTGCTGCTGGGTCAGCTCTGGGACCGTGAGGGCACGGTACTGCTGCGAGC

CACGCGACGTCAGTGGGGCGAAACCGATCATGAAGAAGTGGAGACGCGGGAACGGGATCAAGTTCACGGCGA
GCTTGCGGAGATCCGAGTTCAGCTGACCTGGGAAACGCAGACACGTGGTGATGCCCGACATG

462

TTTCATCCATACCCTCACCCGTGTACCAGTGCAAGAAAGCCTTGCGACGGAACATGGCCGTGAACTGCTCGGA
CACACGCTTGAACATCTCCTGGATGGCAGTCGAGTTACCGATGAACGTGGTACTCATCTTGAGACCCTTAGGCG
GGATGTCACACACGCTGGCCTTGATGTTGTTGGGGATCCATTCAACGAAGTACGAGGAGTTCTTGTTCTGCACG
TTCAGCATCTGTTCATCGACTTCCTTGGTACTCATGCGGCCGCGGAACATACAGGCAGCAGTTAAATAGCGACC
GTGGCGGGGATCAGCAGCGCACATCATATTCTTGGCGTCAAACTGCTGCTGGGTCAGCTCAGGGACGGTGAGA
GCACGGTACTGCTGCGAGCCACGAGAGGTCAGAGGCGCGAAACCAATCATGAAGAAGTGGAGGCGCGGGAAC
GGAATCAGGTTCACGGCCAGCTTACGCAGATCGGAG

A.3.3

Table A1: Two-way ANOVA analysis for the mean number of *Phytophthora* species isolated from all the 25 river sites with bait type and site interaction (includes data from sites and bait type that had no *Phytophthora* recovery)

Source of variation	d.f	s.s	m.s	v.r	P-value
Bait type	2	13.5556	6.7778	17.73	**<0.001****
Site	24	32.8889	1.3704	3.59	**<0.001****
Bait type. site	48	19.7778	0.4120	1.08	0.359
Residual	150	57.3333	0.3822		

** highly significantly different (P≤0.005)

Table A2: One-way ANOVA results for the mean number of *Phytophthora* species isolated from 22 river sites (excluding three sites that had no *Phytophthora* recovery)

Source of variation	d.f	s.s	m.s	F-value	P-value
River sites	21	23.42	1.1152	2.165	**0.00358****
residual	176	90.67	0.5152		

** highly significantly different (P≤0.005)

Table A3: Tukey's honestly significant difference (HSD) for comparison of the number of *Phytophthora* species for each pair of river sites (excluding three sites that had no *Phytophthora* recovery).

Linear hypothesis	Estimate std.	Error	t value	P value
Ashburton2 - Ashburton1 == 0	0.3333	0.3383	0.985	1.0000
Ashburton3 - Ashburton1 == 0	-0.2222	0.3383	-0.657	1.0000
Ashburton5 - Ashburton1 == 0	0.6667	0.3383	1.97	0.9339
Ashburton6 - Ashburton1 == 0	0.1111	0.3383	0.328	1.0000
Halswel1 - Ashburton1 == 0	0.5556	0.3383	1.642	0.9903
Halswel2 - Ashburton1 == 0	0.2222	0.3383	0.657	1.0000
Halswel3 - Ashburton1 == 0	0.7778	0.3383	2.299	0.7760
KaitunaV1 - Ashburton1 == 0	-2.085E-15	0.3383	0	1.0000
KaitunaV2 - Ashburton1 == 0	-2.007E-15	0.3383	0	1.0000
lakehood1 - Ashburton1 == 0	0.2222	0.3383	0.657	1.0000
Kaituna V3 - Ashburton1 == 0	1	0.3383	2.956	0.3024
PricesV1 - Ashburton1 == 0	0.5556	0.3383	1.642	0.9903
PricesV2 - Ashburton1 == 0	0.2222	0.3383	0.657	1.0000
PricesV3 - Ashburton1 == 0	-0.2222	0.3383	-0.657	1.0000
PricesV4 - Ashburton1 == 0	0.7778	0.3383	2.299	0.7745
Selwyn1 - Ashburton1 == 0	0.5556	0.3383	1.642	0.9901
Selwyn2 - Ashburton1 == 0	-0.2222	0.3383	-0.657	1.0000

Table A3 continued

Linear hypothesis	Estimate std.	Error	t value	P value
Selwyn3 - Ashburton1 == 0	0.2222	0.3383	0.657	1.0000
Selwyn4 - Ashburton1 == 0	0.4444	0.3383	1.314	0.9995
Selwyn5 - Ashburton1 == 0	0.5556	0.3383	1.642	0.9902
Selwyn6 - Ashburton1 == 0	-2.46E-15	0.3383	0	1.0000
Ashburton3 - Ashburton2 == 0	-0.5556	0.3383	-1.642	0.9903
Ashburton5 - Ashburton2 == 0	0.3333	0.3383	0.985	1.0000
Ashburton6 - Ashburton2 == 0	-0.2222	0.3383	-0.657	1.0000
Halswell - Ashburton2 == 0	0.2222	0.3383	0.657	1.0000
Halswell2 - Ashburton2 == 0	-0.1111	0.3383	-0.328	1.0000
Halswell3 - Ashburton2 == 0	0.4444	0.3383	1.314	0.9995
KaitunaV1 - Ashburton2 == 0	-0.3333	0.3383	-0.985	1.0000
KaitunaV2 - Ashburton2 == 0	-0.3333	0.3383	-0.985	1.0000
lakehood1 - Ashburton2 == 0	-0.1111	0.3383	-0.328	1.0000
Kaituna V3 - Ashburton2 == 0	0.6667	0.3383	1.97	0.9326
PricesV1 - Ashburton2 == 0	0.2222	0.3383	0.657	1.0000
PricesV2 - Ashburton2 == 0	-0.1111	0.3383	-0.328	1.0000
PricesV3 - Ashburton2 == 0	-0.5556	0.3383	-1.642	0.9902
PricesV4 - Ashburton2 == 0	0.4444	0.3383	1.314	0.9995
Selwyn1 - Ashburton2 == 0	0.2222	0.3383	0.657	1.0000
Selwyn2 - Ashburton2 == 0	-0.5556	0.3383	-1.642	0.9903
Selwyn3 - Ashburton2 == 0	-0.1111	0.3383	-0.328	1.0000
Selwyn4 - Ashburton2 == 0	0.1111	0.3383	0.328	1.0000
Selwyn5 - Ashburton2 == 0	0.2222	0.3383	0.657	1.0000
Selwyn6 - Ashburton2 == 0	-0.3333	0.3383	-0.985	1.0000
Ashburton5 - Ashburton3 == 0	0.8889	0.3383	2.627	0.5347
Ashburton6 - Ashburton3 == 0	0.3333	0.3383	0.985	1.0000
Halswell1 - Ashburton3 == 0	0.7778	0.3383	2.299	0.7741
Halswell2 - Ashburton3 == 0	0.4444	0.3383	1.314	0.9995
Halswell3 - Ashburton3 == 0	1	0.3383	2.956	0.3018
KaitunaV1 - Ashburton3 == 0	0.2222	0.3383	0.657	1.0000
KaitunaV2 - Ashburton3 == 0	0.2222	0.3383	0.657	1.0000
lakehood1 - Ashburton3 == 0	0.4444	0.3383	1.314	0.9995
KaitunaV3- Ashburton3 == 0	1.222	0.3383	3.612	**0.0566 ***
PricesV1 - Ashburton3 == 0	0.7778	0.3383	2.299	0.7743
PricesV2 - Ashburton3 == 0	0.4444	0.3383	1.314	0.9995
PricesV3 - Ashburton3 == 0	1.249E-15	0.3383	0	1.0000
PricesV4 - Ashburton3 == 0	1	0.3383	2.956	0.3025
Selwyn1 - Ashburton3 == 0	0.7778	0.3383	2.299	0.7760
Selwyn2 - Ashburton3 == 0	1.471E-15	0.3383	0	1.0000
Selwyn3 - Ashburton3 == 0	0.4444	0.3383	1.314	0.9995
Selwyn4 - Ashburton3 == 0	0.6667	0.3383	1.97	0.9331
Selwyn5 - Ashburton3 == 0	0.7778	0.3383	2.299	0.7758
Selwyn6 - Ashburton3 == 0	0.2222	0.3383	0.657	1.0000
Ashburton6 - Ashburton5 == 0	-0.5556	0.3383	-1.642	0.9901
Halswell1 - Ashburton5 == 0	-0.1111	0.3383	-0.328	1.0000
Halswell2 - Ashburton5 == 0	-0.4444	0.3383	-1.314	0.9995

Table A3 continued

Linear hypothesis	Estimate std.	Error	t value	P value
Halswell3 - Ashburton5 == 0	0.1111	0.3383	0.328	1.0000
KaitunaV1 - Ashburton5 == 0	-0.6667	0.3383	-1.97	0.9330
KaitunaV2 - Ashburton5 == 0	-0.6667	0.3383	-1.97	0.9339
LakeHood1 - Ashburton5 == 0	-0.4444	0.3383	-1.314	0.9995
KaitunaV3- Ashburton5 == 0	0.3333	0.3383	0.985	1.0000
PricesV1 - Ashburton5 == 0	-0.1111	0.3383	-0.328	1.0000
PricesV2 - Ashburton5 == 0	-0.4444	0.3383	-1.314	0.9995
PricesV3 - Ashburton5 == 0	-0.8889	0.3383	-2.627	0.5366
PricesV4 - Ashburton5 == 0	0.1111	0.3383	0.328	1.0000
Selwyn1 - Ashburton5 == 0	-0.1111	0.3383	-0.328	1.0000
Selwyn2 - Ashburton5 == 0	-0.8889	0.3383	-2.627	0.5350
Selwyn3 - Ashburton5 == 0	-0.4444	0.3383	-1.314	0.9995
Selwyn4 - Ashburton5 == 0	-0.2222	0.3383	-0.657	1.0000
Selwyn5 - Ashburton5 == 0	-0.1111	0.3383	-0.328	1.0000
Selwyn6 - Ashburton5 == 0	-0.6667	0.3383	-1.97	0.9331
Halswell1 - Ashburton6 == 0	0.4444	0.3383	1.314	0.9995
Halswell2 - Ashburton6 == 0	0.1111	0.3383	0.328	1.0000
Halswell3 - Ashburton6 == 0	0.6667	0.3383	1.97	0.9330
KaitunaV1 - Ashburton6 == 0	-0.1111	0.3383	-0.328	1.0000
KaitunaV2 - Ashburton6 == 0	-0.1111	0.3383	-0.328	1.0000
lakehood1 - Ashburton6 == 0	0.1111	0.3383	0.328	1.0000
Kaituna V3 - Ashburton6 == 0	0.8889	0.3383	2.627	0.5341
PricesV1 - Ashburton6 == 0	0.4444	0.3383	1.314	0.9995
PricesV2 - Ashburton6 == 0	0.1111	0.3383	0.328	1.0000
PricesV3 - Ashburton6 == 0	-0.3333	0.3383	-0.985	1.0000
PricesV4 - Ashburton6 == 0	0.6667	0.3383	1.97	0.9324
Selwyn1 - Ashburton6 == 0	0.4444	0.3383	1.314	0.9995
Selwyn2 - Ashburton6 == 0	-0.3333	0.3383	-0.985	1.0000
Selwyn3 - Ashburton6 == 0	0.1111	0.3383	0.328	1.0000
Selwyn4 - Ashburton6 == 0	0.3333	0.3383	0.985	1.0000
Selwyn5 - Ashburton6 == 0	0.4444	0.3383	1.314	0.9995
Selwyn6 - Ashburton6 == 0	-0.1111	0.3383	-0.328	1.0000
Halswell2 - Halswell1 == 0	-0.3333	0.3383	-0.985	1.0000
Halswell3 - Halswell1 == 0	0.2222	0.3383	0.657	1.0000
KaitunaV1 - Halswell1 == 0	-0.5556	0.3383	-1.642	0.9901
KaitunaV2 - Halswell1 == 0	-0.5556	0.3383	-1.642	0.9901
LakeHood1 - Halswell1 == 0	-0.3333	0.3383	-0.985	1.0000
KaitunaV3- Halswell1 == 0	0.4444	0.3383	1.314	0.9995
PricesV1 - Halswell1 == 0	2.998E-15	0.3383	0	1.0000
PricesV2 - Halswell1 == 0	-0.3333	0.3383	-0.985	1.0000
PricesV3 - Halswell1 == 0	-0.7778	0.3383	-2.299	0.7757
PricesV4 - Halswell1 == 0	0.2222	0.3383	0.657	1.0000
Selwyn1 - Halswell1 == 0	2.887E-15	0.3383	0	1.0000
Selwyn2 - Halswell1 == 0	-0.7778	0.3383	-2.299	0.7755
Selwyn3 - Halswell1 == 0	-0.3333	0.3383	-0.985	1.0000

Table A3 continued

Linear hypothesis	Estimate std.	Error	t value	P value
Selwyn4 - Halswell1 == 0	-0.1111	0.3383	-0.328	1.0000
Selwyn5 - Halswell1 == 0	2.776E-15	0.3383	0	1.0000
Selwyn6 - Halswel1 == 0	-0.5556	0.3383	-1.642	0.9901
Halswel3 - Halswell2 == 0	0.5556	0.3383	1.642	0.9903
KaitunaV1 - Halswell2 == 0	-0.2222	0.3383	-0.657	1.0000
KaitunaV2 - Halswell2 == 0	-0.2222	0.3383	-0.657	1.0000
LakeHood1 - Halswell2 == 0	-7.772E-16	0.3383	0	1.0000
KaitunaV3- Halswell2 == 0	0.7778	0.3383	2.299	0.7751
PricesV1 - Halswell2 == 0	0.3333	0.3383	0.985	1.0000
PricesV2 - Halswell2 == 0	7.494E-16	0.3383	0	1.0000
PricesV3 - Halswell2 == 0	-0.4444	0.3383	-1.314	0.9995
PricesV4 - Halswell2 == 0	0.5556	0.3383	1.642	0.9902
Selwyn1 - Halswell2 == 0	0.3333	0.3383	0.985	1.0000
Selwyn2 - Halswell2 == 0	-0.4444	0.3383	-1.314	0.9995
Selwyn3 - Halswell2 == 0	1.388E-16	0.3383	0	1.0000
Selwyn4 - Halswell2 == 0	0.2222	0.3383	0.657	1.0000
Selwyn5 - Halswell2 == 0	0.3333	0.3383	0.985	1.0000
Selwyn6 - Halswell2 == 0	-0.2222	0.3383	-0.657	1.0000
KaitunaV1 - Halswell3 == 0	-0.7778	0.3383	-2.299	0.7733
KaitunaV2 - Halswell3 == 0	-0.7778	0.3383	-2.299	0.7744
LakeHood1 - Halswell3 == 0	-0.5556	0.3383	-1.642	0.9902
KaitunaV3- Halswell3 == 0	0.2222	0.3383	0.657	1.0000
PricesV1 - Halswell3 == 0	-0.2222	0.3383	-0.657	1.0000
PricesV2 - Halswell3 == 0	-0.5556	0.3383	-1.642	0.9904
PricesV3 - Halswell3 == 0	-1	0.3383	-2.956	0.3020
PricesV4 - Halswell3 == 0	-1.665E-15	0.3383	0	1.0000
Selwyn1 - Halswell3 == 0	-0.2222	0.3383	-0.657	1.0000
Selwyn2 - Halswell3 == 0	-1	0.3383	-2.956	0.3025
Selwyn3 - Halswell3 == 0	-0.5556	0.3383	-1.642	0.9901
Selwyn4 - Halswell3 == 0	-0.3333	0.3383	-0.985	1.0000
Selwyn5 - Halswell3 == 0	-0.2222	0.3383	-0.657	1.0000
Selwyn6 - Halswell3 == 0	-0.7778	0.3383	-2.299	0.7750
KaitunaV2 - KaitunaV1 == 0	7.835E-17	0.3383	0	1.0000
LakeHood1 - KaitunaV1 == 0	0.2222	0.3383	0.657	1.0000
KaitunaV3- KaitunaV1 == 0	1	0.3383	2.956	0.3008
PricesV1 - KaitunaV1 == 0	0.5556	0.3383	1.642	0.9901
PricesV2 - KaitunaV1 == 0	0.2222	0.3383	0.657	1.0000
PricesV3 - KaitunaV1 == 0	-0.2222	0.3383	-0.657	1.0000
PricesV4 - KaitunaV1 == 0	0.7778	0.3383	2.299	0.7749
Selwyn1 - KaitunaV1 == 0	0.5556	0.3383	1.642	0.9902
Selwyn2 - KaitunaV1 == 0	-0.2222	0.3383	-0.657	1.0000
Selwyn3 - KaitunaV1 == 0	0.2222	0.3383	0.657	1.0000
Selwyn4 - KaitunaV1 == 0	0.4444	0.3383	1.314	0.9995
Selwyn5 - KaitunaV1 == 0	0.5556	0.3383	1.642	0.9903
Selwyn6 - KaitunaV1 == 0	-3.748E-16	0.3383	0	1.0000

Table A3 continued

Linear hypothesis	Estimate std.	Error	t value	P value
LakeHood1 - KaitunaV2 == 0	0.2222	0.3383	0.657	1.0000
KaitunaV3- KaitunaV2 == 0	1	0.3383	2.956	0.3017
PricesV1 - KaitunaV2 == 0	0.5556	0.3383	1.642	0.9902
PricesV2 - KaitunaV2 == 0	0.2222	0.3383	0.657	1.0000
PricesV3 - KaitunaV2 == 0	-0.2222	0.3383	-0.657	1.0000
PricesV4 - KaitunaV2 == 0	0.7778	0.3383	2.299	0.7756
Selwyn1 - KaitunaV2 == 0	0.5556	0.3383	1.642	0.9902
Selwyn2 - KaitunaV2 == 0	-0.2222	0.3383	-0.657	1.0000
Selwyn3 - KaitunaV2 == 0	0.2222	0.3383	0.657	1.0000
Selwyn4 - KaitunaV2 == 0	0.4444	0.3383	1.314	0.9995
Selwyn5 - KaitunaV2 == 0	0.5556	0.3383	1.642	0.9902
Selwyn6 - KaitunaV2 == 0	-4.532E-16	0.3383	0	1.0000
KaitunaV3- LakeHood1 == 0	0.7778	0.3383	2.299	0.7754
PricesV1 - LakeHood1 == 0	0.3333	0.3383	0.985	1.0000
PricesV2 - LakeHood1 == 0	1.527E-15	0.3383	0	1.0000
PricesV3 - LakeHood1 == 0	-0.4444	0.3383	-1.314	0.9995
PricesV4 - LakeHood1 == 0	0.5556	0.3383	1.642	0.9903
Selwyn1 - LakeHood1 == 0	0.3333	0.3383	0.985	1.0000
Selwyn2 - LakeHood1 == 0	-0.4444	0.3383	-1.314	0.9995
Selwyn3 - LakeHood1 == 0	9.159E-16	0.3383	0	1.0000
Selwyn4 - LakeHood1 == 0	0.2222	0.3383	0.657	1.0000
Selwyn5 - LakeHood1 == 0	0.3333	0.3383	0.985	1.0000
Selwyn6 -LakeHood1 == 0	-0.2222	0.3383	-0.657	1.0000
PricesV1 - KaitunaV3== 0	-0.4444	0.3383	-1.314	0.9995
PricesV2 - KaitunaV3== 0	-0.7778	0.3383	-2.299	0.7761
PricesV3 - KaitunaV3== 0	-1.222	0.3383	-3.612	**0.0562 ***
PricesV4 - KaitunaV3== 0	-0.2222	0.3383	-0.657	1.0000
Selwyn1 KaitunaV3== 0	-0.4444	0.3383	-1.314	0.9995
Selwyn2 - KaitunaV3== 0	-1.222	0.3383	-3.612	**0.0562 ***
Selwyn3 - KaitunaV3== 0	-0.7778	0.3383	-2.299	0.7751
Selwyn4 KaitunaV3== 0	-0.5556	0.3383	-1.642	0.9903
Selwyn5 - KaitunaV3== 0	-0.4444	0.3383	-1.314	0.9995
Selwyn6 - KaitunaV3== 0	-1	0.3383	-2.956	0.3030
PricesV2 - PricesV1 == 0	-0.3333	0.3383	-0.985	1.0000
PricesV3 - PricesV1 == 0	-0.7778	0.3383	-2.299	0.7752
PricesV4 - PricesV1 == 0	0.2222	0.3383	0.657	1.0000
Selwyn1 - PricesV1 == 0	-1.11E-16	0.3383	0	1.0000
Selwyn2 - PricesV1 == 0	-0.7778	0.3383	-2.299	0.7747
Selwyn3 - PricesV1 == 0	-0.3333	0.3383	-0.985	1.0000
Selwyn4 - PricesV1 == 0	-0.1111	0.3383	-0.328	1.0000
Selwyn5 - PricesV1 == 0	-2.22E-16	0.3383	0	1.0000
Selwyn6 - PricesV1 == 0	-0.5556	0.3383	-1.642	0.9904
PricesV3 - PricesV2 == 0	-0.4444	0.3383	-1.314	0.9995
PricesV4 - PricesV2 == 0	0.5556	0.3383	1.642	0.9902

Table A3 continued

Linear hypothesis	Estimate std.	Error	t value	P value
Selwyn1 - PricesV2 == 0	0.3333	0.3383	0.985	1.0000
Selwyn2 - PricesV2 == 0	-0.4444	0.3383	-1.314	0.9995
Selwyn3 - PricesV2 == 0	-6.106E-16	0.3383	0	1.0000

* significantly different (P=0.1)

Table A4: Two-way ANOVA results for the mean number of *Phytophthora* species (diversity) isolated from 22 river sites (excluding three sites and bait type that had no *Phytophthora* recovery)

Source of variation	d.f	s.s	m.s	v.r	P-value
Bait_type ignoring Site	2	3.6155	1.8078	6.45	**0.004** **
Bait_type eliminating Site	2	3.5880	1.7940	6.40	**0.004** **
Site ignoring Bait_type	21	5.6695	0.2700	0.96	0.523
Site eliminating Bait_type	21	5.6419	0.2687	0.96	0.528
Bait_type.Site	29	6.0192	0.2076	0.74	0.800
Residual	41	11.500	0.2805		

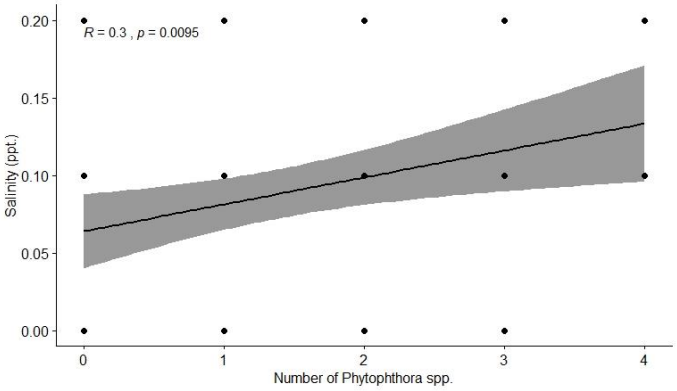
** highly significantly different (P≤0.005)

Table A5: Two-way ANOVA results for the mean number of *Phytophthora* isolates recovered from 22 river sites (excluding three sites and bait type that had no *Phytophthora* recovery)

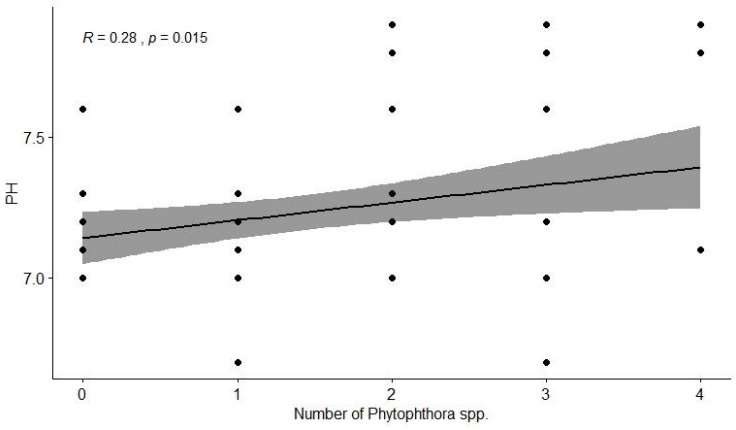
Source of variation	d.f	s.s	m.s	v.r	P-value
Bait_type ignoring Site	2	6.4242	3.2121	5.16	**0.010***
Bait_type eliminating Site	2	5.9467	2.9734	4.78	**0.014***
Site ignoring Bait_type	21	13.3893	0.6376	1.03	0.458
Site eliminating Bait_type	21	12.9119	0.6149	0.99	0.496
Bait_type.Site	29	10.4937	0.3619	0.58	0.935
Residual	41	25.5000	0.6220		

*significantly different (P≤0.05)

1)



2)



3)

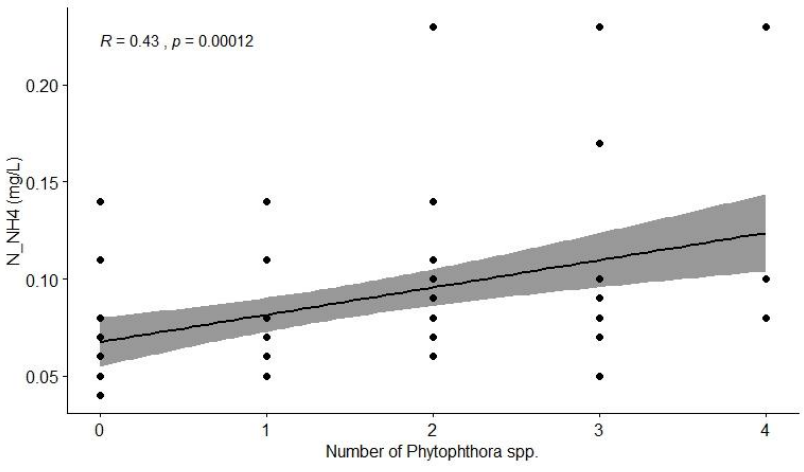
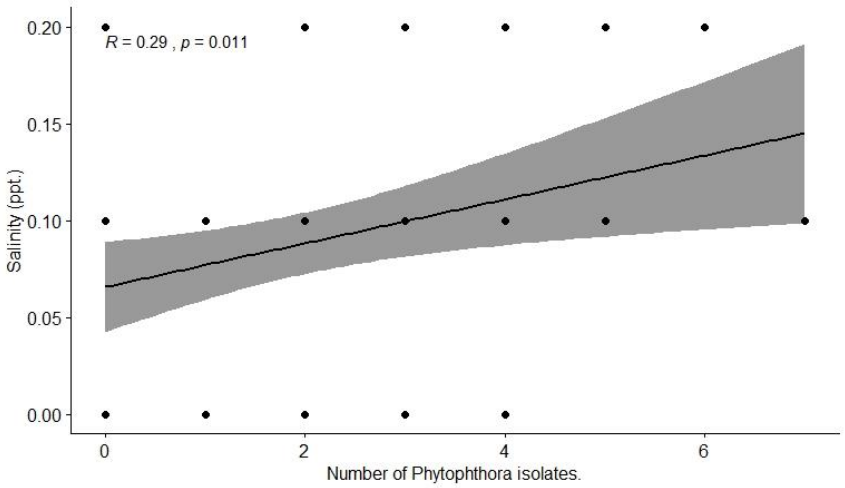
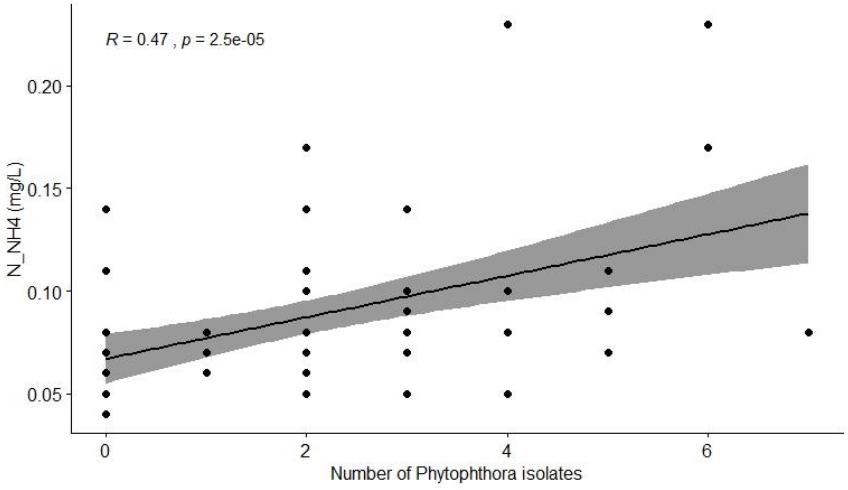


Figure A6: Pearson's correlation relationship between the number of *Phytophthora* spp. recovered and (1) the water salinity in the 22 sites, (2) the water pH in the 22 sites, (3) ammonium nitrogen levels in the water samples from 22 sites.



1)



2)

Figure A7: Pearson's correlation relationship between number of *Phytophthora* isolates recovered and (1) the water salinity in the 22 sites, (2) ammonium nitrogen in the water samples from 22 sites.

Table A7: One-way ANOVA results for the mean number of *Phytophthora* species isolated from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting using three bait types (*R. arboreum, Pi. radiata and Ce. deodara*).

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
River sites	1	2.083	2.083	1.47	0.253
residual	10	14.167	1.417		

Table A8: Two-way ANOVA results for the mean number of *Phytophthora* species isolated from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting using three bait types (*R. arboreum, Pi. radiata and Ce. deodara*) compared with bait type and season.

Source of variation	**d.f**	**s.s**	**m.s**	**v.r**	**P-value**
Bait	2	6.5000	3.2500	5.57	0.043*
Season	1	4.0833	4.0833	7.00	0.038*
Bait. Season	2	2.1667	1.0833	1.86	0.236
Residual	6	3.5000	0.5833		

*significantly different (P≤0.05)

Table A9: Two-way ANOVA results for the mean number of *Phytophthora* species isolated from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting using three bait types (*R. arboreum, Pi. radiata and Ce. deodara*) compared with bait type and season with salinity as the covariate.

Source of variation	**d.f**	**s.s**	**m.s**	**v.r**	**P-value**
Bait	2	6.5000	3.2500	5.57	0.043*
Season	1	4.0833	4.0833	7.00	0.038*
Bait. Season	2	2.1667	1.0833	1.86	0.236
Residual	6	3.5000	0.5833		

*significantly different (P≤0.05)

Table A10: Two-way ANOVA results for the mean number of *Phytophthora* species isolated from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting using three bait types (*R. arboreum, Pi. radiata and Ce. deodara*) compared with bait type and season with pH as the covariate.

Source of variation	**d.f**	**s.s**	**m.s**	**v.r**	**Cov. ef**	**P-value**
Bait	2	6.5000	3.2500	4.69	1.00	0.071
Season	1	2.1258	2.1258	3.07	0.43	0.140
Bait. Season	2	2.1667	1.0833	1.56	1.00	0.297
Covariate	1	0.0333	0.0333	0.05		0.835
Residual	5	3.4667	0.6933		0.84	

Table A11: Two-way ANOVA results for mean number of *Phytophthora* isolates recovered from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting using three bait types (*R. arboreum, Pi. radiata and Ce. deodara*) compared with bait type and season.

Source of variation	**d.f**	**s.s**	**m.s**	**v.r**	**P-value**
Bait	2	51.167	25.583	17.06	0.003
Season	1	0.000	0.000	0.00	1.000
Bait. Season	2	6.500	3.250	2.17	0.196
Residual	6	9.000	1.500		

Table A12: Two-way ANOVA results for mean number of *Phytophthora* isolates recovered from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting using three bait types (*R. arboreum, Pi. radiata and Ce. deodara*) Vs bait type and season with pH as the covariate.

Source of variation	**d.f**	**s.s**	**m.s**	**v.r**	**Cov.ef**	**P-value**
Bait	2	51.473	25.736	15.20	1.00	0.007
Season	1	0.000	0.000	0.00	0.43	1.000
Bait. Season	2	6.500	3.250	1.92	1.00	0.241
Covariate	1	0.533	0.533	0.31		0.599
Residual	5	8.467	1.693		0.89	

Table A13: Bonferroni test results for the difference in the mean number of *Phytophthora* isolates recovered from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting on three bait types *R. arboreum, Pi. radiata and Ce. deodara* with pH as the covariate

Bait type	Mean number of isolates	Difference
R. arboreum	0.750	b
Pi. radiata	1.000	a
Ce. deodara	5.250	a

Table A14: Two-way ANOVA results for mean number of *Phytophthora* isolates recovered from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting using three bait types (*R. arboreum, Pi. radiata and Ce. deodara*) Vs bait type and season with pH, salinity, water temperature and nitrogen (ammonium and nitrate) as the covariate.

Source of variation	**d.f**	**s.s**	**m.s**	**v.r**	**Cov.ef**	**P-value**
Bait	2	53.010	26.505	22.50	1.00	0.003
Season	1	0.000	0.000	0.00	0.41	1.000
Bait. Season	2	6.500	3.250	2.76	1.00	0.516
Covariate	1	3.109	3.109	2.64		0.165
Residual	5	5.891	1.178		1.27	

Table A15: Bonferroni test results for the difference in the mean number of *Phytophthora* isolates recovered from the Halswell River (site 1 and 2) in summer and autumn laboratory baiting on three bait types *R. arboreum, Pi. radiata and Ce. deodara* with pH, salinity, water temperature and nitrogen (ammonium and nitrate) as the covariate.

Bait type	Mean number of isolates	Difference
R. arboreum	5.250	b
Pi. radiata	0.750	a
Ce. deodara	1.000	a

Table A16: One-way ANOVA results for mean number of *Phytophthora* isolates isolated from different river baiting sites on 3 leaf bait types (*R. arboreum, Pi. Radiata, Ce. deodara*)

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
Bait types	2	23.04	11.520	15	0.000008***
residual	195	149.77	0.768		

*** highly significantly different (P≤0.0005)

Table A17: One-way ANOVA results for mean number of *Phytophthora* species isolated from different river baiting sites on 3 leaf bait types (*R. arboreum, Pi. Radiata, Ce. deodara*)

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
Bait types	2	15.40	7.702	15.22	0.0000007 ***
residual	195	98.68	0.506		

*** highly significantly different (P≤0.0005)

Appendix A.4: Supplementary material for chapter 4

A.4.1

Table A1: Experimental design for pathogenicity test of *Phytophthora* spp. isolates using agar plugs on lupin seedlings grown in sterile water (Section 4.2.1). Five replicates for each isolate were arranged in a completely randomised design. (1 = *Ph. gonapodyides,* 2 = *Ph. chlamydospora,* 3 = *Phytophthora* sp. LS-2018c strain CL 181; NC= negative control, a= sterile water, b = sterile deionised water, :1 = replication number).

1:5b	3:4a	2:4b	3:2a	1:4a	1:1b	3:3b
3:2b	2:2b	1:2a	1:4b	2:5a	2:5b	
2:4a	1:5a	3:1a	3:1b	NCb	3:2b	2:1a-
3:5a	3:5a	1:3a	3:4b	1:2b	1:3b	
NCa	2:2a	3:5b	2:1b	2:3a	3:3a	1:1a

Table A2: Experimental design for pathogenicity test of *Phytophthora* spp. isolates using agar plugs on lupin seedlings grown in sterile soil extract solution (Section 4.2.2), sterile paper towels (Section 4.2.3) and vermiculite (Section 4.2.4). Five replicates of the three *Phytophthora* spp. isolates (1 = *Ph. gonapodyides,* 2 = *Ph. chlamydospora,* 3 = *Phytophthora* sp. LS-2018c strain CL 181), positive control (PC; *Ph. cactorum*) and negative control (NC; no agar plug) were arranged in a randomised block design, :1 = replication number).

Block 1			Block 2		
3:1	PC:1	NC:1	PC:2	2:1	NC:2
2:1	1:1		1:2	3:2	

Block 3			Block 4		
2:3	NC:3	3:3	1:4	PC:4	2:4
PC:3	1:3		NC:4	3:4	

Block 5

NC:5 PC:4 2:5

1:5 3:5

A.4.2

Table A1: One-way ANOVA results for mean pathogenicity score obtained on *Lupinus angustifolius* (Blue Lupin) seedling inoculated with four *Phytophthora* spp. isolates, being *Phytophthora* sp. LS-2018c strain CL 181*, Ph. chlamydospora*, *Ph. gonapodyides* and *Ph. cactorum* (positive control).

Source of variation	**d.f**	**s.s**	**m.s**	**F-value**	**P-value**
Treatment	3	1.50	0.50	0.90	0.478
residual	9	5.00	0.5556		

Table A2: Multiple comparison of mean pathogenicity score between the four *Phytophthora* spp. isolates tested using Bonferroni test. Where, 1= *Ph. gonapodyides,* 2= *Ph. chlamydospora* and 3= *Phytophthora* sp. LS-2018c strain CL 181, NC= negative control, PC = *Ph. cactorum* (positive control).

Comparison	**Difference**	**Lower 95%**	**Upper 95 %**	**t**	**Significant**
NC vs 1	-1.0000	-2.611	0.6111	-2.128	No
NC vs 2	-1.5000	-3.111	0.1111	-3.192	No
1 vs 2	-0.5000	-2.111	1.1111	-1.064	No
1 vs 3	-0.7500	-2.361	0.8611	-1.596	No
1 vs PC	-0.7500	-2.361	0.8611	-1.596	No
2 vs 3	-0.2500	-1.861	1.3611	-0.532	No
2 vs PC	-0.2500	-1.861	1.3611	-0.532	No
3 vs PC	0.0000	-1.611	1.6111	0.000	No